ZINC FINGER PROTEINS FOR TREATING PRION DISEASE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Application 63/564,791, filed on March 13, 2024, U.S. Provisional Application 63/643,900, filed on May 7, 2024. The contents of the aforementioned provisional applications are incorporated herein by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing that has been submitted electronically in XML format. The Sequence Listing is hereby incorporated by reference in its entirety. The attached sequence listing comprises 1500 sequences. The attached sequence listing is 1.46 MB in size and has the file name "91355.11516.xml" and has a production date of March 12, 2025.

BACKGROUND OF THE INVENTION

[0003] Prion disease refers to a group of progressive neurodegenerative disorders that affect both humans and animals. These disorders are characterized by the accumulation of a misfolded isoform of the prion protein (PrP Scrapie; PrP^{Sc}) leading to spongiform changes to the brain associated with neuronal loss and gliosis. The term prion, short for proteinaceous infectious particle, refers to the protein-only nature of these pathogenic isoforms. The abnormally folded protein (PrP^{Sc}) can subsequently bind and convert the abundantly expressed physiological form of the prion protein (cellular PrP; PrP^{C}) to the disease-causing isoform PrP^{Sc}. This phenomenon is known as self-templating. Although identical in protein sequence, PrP^{Sc} is drastically different from PrP^{C} biophysically in terms of solubility, structure, and stability (Riesner, *Brit Med Bull.* (2003) 66:21-33). Propagation of the PrP^{Sc} isoform is followed by aggregation, causing neuronal cell death in the central nervous system. Human prion diseases can be genetic (accounting for 10-15% of cases), sporadic or acquired and include Creutzfeldt-Jakob Disease (CJD), Gerstmann-Straussler-Scheinker Syndrome (GSS), Fatal Familial Insomnia (FFI), and Kuru. In humans, prion disease impairs brain function, causing progressive cognitive decline and abnormal movements. Prion disease is always fatal, and typically results in death within a few months to several years of onset of illness.

[0004] The precise function of PrP is still controversial in the field, but among many phenotypes, PrP has been hypothesized to play a role in neurogenesis and neuroprotection, circadian rhythm, myelin maintenance, epithelial to mesenchymal transition

(EMT) and long-term potentiation (LTP). In addition to the familial forms, prion disease can also be sporadic or acquired. People with sporadic prion disease have no family history of the disease or identifiable mutation in the *PRNP* gene. Sporadic prion disease occurs when PrP^{C} is spontaneously transformed into PrP^{Sc}. Sporadic forms of prion disease include sporadic CJD (sCJD), sporadic fatal insomnia (sFI), and variably protease-sensitive prionopathy (VPSPr). Acquired prion disease results from exposure to PrP^{Sc} from an outside source. For example, variant CJD (vCJD) is a form of acquired prion disease resulting from consumption of beef products containing PrP^{Sc} from cattle with prion disease. In cattle, this form of the disease is known as bovine spongiform encephalopathy (BSE) or "mad cow disease." Another example of an acquired human prion disease is Kuru, which was identified in the South Fore population in Papua New Guinea. Kuru was transmitted when individuals ate affected human tissue during cannibalistic funeral rituals.

[0005] Reduction of PrP expression is a therapeutic strategy supported by numerous genetic proof of concept studies as well as by the *in vivo* efficacy of PrP-lowering antisense oligonucleotides (ASOs) shown to prolong the survival of prion-infected mice. *See, e.g.,* Büeler et al., *Cell* (1993) 73(7):1339-47; Büeler et al., *Mol Med Camb Mass.* (1994) 1(1):19-30; Fischer et al., *EMBO J.* (1996) 15(6):1255-64; Mallucci et al., *Science* (2003) 302(5646):871-4; and Safar et al., *J Gen Virol.* (2005) 86(Pt 10):2913-23, Minikel et al., *Nucleic Acids Res.* (2020) 10.1093/nar/gkaa616. While ASOs achieving 50% PrP knockdown have been shown to delay the onset of prion disease in mice by more than two-fold, greater levels of PrP knockdown may offer further therapeutic benefit. Distribution of PrP knockdown beyond what is achievable with ASOs may also be important for efficacy. Thus, there remains a need for an effective treatment of prion disease by targeting PrP expression.

[0006] International Patent Publication WO 2021067864 (which is herein incorporated by reference in its entirety) shows prion protein gene (*PRNP* gene) repressing activity of 36 ZFRs selected from a library of 384 ZFRs directed to target the mouse *PRNP* gene. As generally is the case with genomic therapies targeting the brain, delivering therapeutic reagents across the blood-brain barrier is a significant challenge. Indeed, the clinical potential of AAV vectors as gene delivery tools for neurological diseases is constrained by the low capacity of natural AAV capsids to cross the blood-brain barrier (BBB). As a result, delivery of AAV vectors to the central nervous system (CNS) can require high vector doses or invasive local delivery (injection), both associated with significant risks of vector-induced toxicity. Further, injection relies on diffusion and does not effectively distribute the AAV vector

throughout the brain. To overcome these limitations, multiple groups have attempted to generate engineered AAV capsids with improved BBB penetration and CNS transduction.

[0007] Moreover, the brain regions targeted by therapeutics may be those most significantly affected in Tauopathies, such as certain cortical regions, the entorhinal cortex, the hippocampus, the cerebellum, the globus pallidus, the thalamus, the midbrain, the caudate, the putamen, the substantia nigra, the pons, and the medulla. ASO therapies and presumably many AAVs therapies are not able to target one or more of these affected regions.

SUMMARY OF THE INVENTIOIN

[0008] Disclosed are zinc finger protein (ZFP) domains that target sites in or near the mammalian (*e.g.*, human, non-human primate, rodent, or murine) *PRNP* gene. The ZFP domains may be fused to a transcription factor to specifically inhibit the mammalian *PRNP* gene at the DNA level. These fusion proteins, also termed zinc finger protein transcription factors (ZFP-TFs), zinc finger protein repressors (ZFP-Rs), or zinc finger repressors (ZFRs), comprise (i) a ZFP domain that binds specifically to a target region in the *PRNP* gene and (ii) a transcription repressor domain or epigenetic silencing domain that reduces the transcription of the gene. These proteins can be used to treat prion disease.

[0009] In one aspect, disclosed are fusion proteins comprising a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a mammalian (*e.g.*, human, non-human primate, rodent or murine) prion protein gene (*PRNP* gene). In some embodiments, the target region of the ZFR is within about 1 kb or 500 bp of a transcription start site (TSS) in the *PRNP* gene. In some embodiments, the fusion protein may comprise one or more (*e.g.*, two, three, four, five, or six) zinc fingers and it optionally represses expression of the *PRNP* gene by at least about 40%, 75%, 90%, 95%, or 99% with no or minimal detectable off-target binding or activity. Nonlimiting examples of zinc finger domains are shown in Tables 1 and 3. In some embodiments, the fusion protein comprises one or more recognition helix sequences shown in Tables 1 and 3. In further embodiments, the fusion protein comprises some or all the recognition helix sequences from a single row of Tables 1 and 3, with or without the indicated backbone mutation(s). In certain embodiments, the fusion protein comprises an amino acid sequence shown in Tables 2 and 4.

[0010] In some embodiments, the transcription repression domain of the fusion protein may comprise a KRAB domain, optionally amino acid sequence of KOX1. In the fusion protein, the ZFP domain may be linked to the transcription repressor domain or epigenetic silencing domain through a peptide linker.

[0011] In another aspect, disclosed is a nucleic acid construct comprising a coding sequence for the fusion protein, wherein the coding sequence is linked operably to a transcription regulatory element, such as a mammalian promoter that is constitutively active or inducible in a brain cell (*e.g.*, a human synapsin I promoter). Disclosed are host cells comprising the nucleic acid construct. The host cell may be, *e.g.*, a human cell, and/or a brain cell or a pluripotent stem cell, wherein the stem cell is optionally an embryonic stem cell or an inducible pluripotent stem cell (iPSC). Disclosed is a recombinant virus (recombinant adeno-associated virus (AAV), preferably a BBB-penetrant AAV, optionally of serotype AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV11, AAVrh10, AAVrh39, AAVrh74, or STAC-BBB (*i.e.*, CNSRCV300 in US Application No.63/606,012), and natural or engineered derivatives thereof.

[0012] In yet another aspect, disclosed is a method of inhibiting expression of prion protein (PrP) in a mammalian brain cell, comprising introducing into the cell a fusion protein, optionally through introduction of a nucleic acid construct (*e.g.*, through a recombinant virus) described herein, thereby inhibiting the expression of PrP in the cell. The mammalian brain cell may be, for example, a human, non-human primate, rodent, or murine cell, and/or may be a neuron, a glial cell, an ependymal cell, or a neuroepithelial cell. In some embodiments, the cell is in the brain of a patient suffering from or at risk of developing prion disease, wherein the prion disease is optionally familial, sporadic, or acquired prion disease, such as Creutzfeldt-Jakob Disease (CJD), sporadic CJD, variant CJD, Gerstmann-Straussler-Scheinker Syndrome (GSS), Fatal Familial Insomnia (FFI), sporadic Fatal Insomnia (sFI), Kuru, or variably protease-sensitive prionopathy (VPSPr).

[0013] Disclosed is a method of treating a neurodegenerative disease in a patient, comprising administering to the patient a recombinant AAV encoding a fusion protein. The neurodegenerative disease may be a prion disease, wherein the prion disease is optionally familial, sporadic, or acquired prion disease, such as CJD, sporadic CJD, variant CJD, GSS, FFI, sFI, Kuru, or VPSPr. In some embodiments, the disease may be a Tauopathy, such as Alzheimer's disease (AD), progressive supranuclear palsy (PSP), frontotemporal dementia (FTD), or corticobasal degeneration (CBD), or chronic traumatic encephalopathy (CTE). In other embodiments, the disease may be a synucleinopathy, such as Parkinson's disease (PD), multiple systems atrophy (MSA), or dementia with Lewy bodies (DLB).

[0014] In some embodiments, the AAV encoding a fusion protein is introduced to the patient via intravenous, intrathecal, intracerebroventrical, intra-cisternal magna, or intrathalamic injection, or injection into any cerebral region.

[0015] Disclosed are fusion proteins, nucleic acid constructs, and recombinant viruses for use in the methods described in, as well as use of the fusion proteins, nucleic acid constructs, recombinant viruses for the manufacture of a medicament in the methods described herein.

A composition comprising: 1) an adeno-associated virus (AAV) capsid protein comprising an amino acid sequence, wherein the amino acid sequence comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the amino acid sequence set forth in any one of SEQ ID NOS: 333, 417, 442, or 673; and 2) an expression construct comprising a coding sequence for a fusion protein, wherein the fusion protein comprises a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a mammalian prion protein gene (*PRNP* gene). In some embodiments, the AAV capsid protein encapsulates the expression construct. In some embodiments, the amino acid sequence comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the amino acid sequence set forth in SEQ ID NO: 333 inserted into SEQ ID. NO: 1497, optionally wherein the insertion is between amino acids 587 and 590. In some embodiments, the AAV capsid protein comprising one or more amino acid sequences selected from the group comprising, consisting, or consisting essentially of SEQ ID NO: 1498, 1499, or 1500. In some embodiments, the AAV capsid protein comprising a VP1 protein comprising a sequence of amino acids set forth in SEQ ID NO: 1498; a VP2 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1499; and a VP3 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1500. In some embodiments, the AAV capsid protein comprising the amino acid sequence having at least 80%, 85%, 90%, 95%, 99% or 100% identity to SEQ ID NO: 1498. In some embodiments, the AAV capsid protein comprising the amino acid sequence having at least 80%, 85%, 90%, 95%, 99% or 100% identity to SEQ ID NO: 1499 or to SEQ ID NO: 1500. In some embodiments, the target region is within about 1 kb or 500 bp of a transcription start site (TSS) in the *PRNP* gene. In some embodiments, the fusion protein represses expression of the *PRNP* gene by at least about 40%, 75%, 90%, 95%, or 99% with no or minimal detectable off-target binding or activity. In some embodiments, the transcription repressor domain comprising a KRAB domain, wherein the KRAB domain optionally is from a human KOX1 protein. In some embodiments, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequence as shown in Tables 1 and 3. In some embodiments, the ZFP domain comprising, consisting, or consisting essentially of DNA-binding recognition helix sequences shown in a single row in Tables 1 and 3. In some embodiments, the ZFP domain comprising, consisting, or consisting essentially of a DNA-

binding recognition helix sequences linked as shown in Tables 2 and 4. In some embodiments, the ZFP domain binds to a Target Sequence shown in Tables 1 and 3. In some embodiments, the fusion protein comprising, consisting, or consisting essentially of a sequence shown in Tables 2 and 4.

[0016] Other features, objectives, and advantages of the invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments and aspects of the invention, is given by way of illustration only, not limitation. Various changes and modification within the scope of the invention will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] **FIG. 1A** is a diagram illustrating site specific targeting of the *PRNP* gene by an engineered zinc finger protein-transcriptional repressor (ZFP-R). Binding of the ZFP-R to the gene locus leads to reduced *PRNP* transcription, which in turn leads to reduced *PRNP* mRNA and prion protein (PrP^C) levels.

[0018] **FIG. 1B** is a schematic showing the packaging of a transgene encoding a Zinc Finger Repressor (ZFR) targeting the *PRNP* gene into an AAV capsid protein STAC-BBB (herein defined as SEQ ID NO: 1498). The packaged ZFR in the AAV capsid protein can be administered to a subject for the treatment of a Tauopathy.

[0019] **FIG. 2** shows human *PRNP* repression activity of 10 ZFP-R candidates selected from a library of 961 ZFP-Rs designed to specifically target the human *PRNP* gene. The y-axis in each graph is human *PRNP* mRNA expression normalized to the mean of two housekeeping genes (*ATP5B* and *EIF4A2*). *PRNP* levels in human neuroblastoma (SK-N-MC) cells were assessed 20-24 hours after being transfected with mRNA encoding different ZFP-Rs. The mRNA dose increases from left to right (3, 10, 30, 100, 300, 1000 ng per transfection). The bars represent the mean of four technical replicates and the error bars represent standard deviation. The numbers below the graphs are the ID numbers for the ZFP-Rs. An enlarged version of the titration scale is shown at the lower right of the figure. The orange bar in each graph represents the *PRNP* levels in a non-treated control.

[0020] **FIG 3** is a panel of graphs representing the 7 ZFP-Rs after several rounds of *in vitro* screening using on- and off-targeting analysis. The top panel shows the *PRNP*-repression activity of ZFP-Rs 31 days after transduction of human iPSC-derived GABAergic neuron cells with the AAV6 serotype. The y-axis in each graph is human *PRNP* mRNA expression normalized to the mean of two housekeeping genes (*ATP5B* and *EIF4A2*). The dark green bars in each graph shows the expression level from the untreated control in each

experiment. The lighter green bars represent the doses delivered, from left to right: 1e3, 3e3, 1e4, 3e4, 1e5, 3e5 vg/cell (MOI). The middle panel shows a whole transcriptome analysis by Affymetrix genechip arrays of human iPSC-derived GABAergic neurons 19 days post transduction with ZFP-Rs delivered using an AAV6 serotype. Cells were transduced at a dose of 1E5 vg/cell (MOI). The bottom panel shows a whole transcriptome analysis by Affymetrix genechip arrays of mouse cortical neurons 7 days post transduction with ZFP-Rs delivered using an AAV6 serotype. Cells were transduced at 3E3 vg/cell (MOI). There was no repression of the mouse *Prnp* gene since the target sites recognized by these ZFP-Rs are only present in human prion gene locus, and they are not conserved with the mouse gene. Numbers shown in red and green indicate the counts of downregulated and upregulated off-target genes, respectively. The yellow circle represents the prion transcript (*PRNP*, middle panel; or *Prnp*, bottom panel). Red and green circles represent downregulated and upregulated off-target genes, respectively, with the gene names labeled adjacent to each circle.

[0021] **FIG. 4A** is a panel of graphs demonstrating tolerability of the top 5 ZFP-Rs delivered to adult wildtype mice using AAV-PHP.B. The bar graphs show RT-qPCR expression level analysis of the mouse *Prnp* gene, neuroinflammatory markers (*Iba1* and *Gfap*), and neuronal markers (*Rbfoxs* and *Tubb3*) for the brainstem three weeks after intravenous tail vein injection. The expression level of each transcript was normalized to house-keeping transcripts (*Atp5b* and *Eif4a2*) and scaled to the mean of the vehicle group. Each bar represents the mean from a treatment group and the error bars represent standard deviation (n=6 animals per group). ZFP-R 89891 showed a significant increase of both neuroinflammatory markers as well as a down-regulation of neuronal markers that indicates potential toxicity. Other ZFP-Rs showed no or minimal changes in neuroinflammatory or neuronal markers. There was no repression of the mouse *Prnp* gene for all ZFP-Rs as expected since the target sites recognized by these ZFP-Rs are only present in human prion gene locus, and they are not conserved with the mouse gene. A one-way ANOVA analysis followed by Dunnett´s multiple comparison test was used to determine statistical significance; P value <0.05 (*), <0.0001 (****), not significant (ns).

[0022] **FIG. 4B** is a line graph showing mouse body weights measured on different study days. Symbols represent the average body weight from each group and the error bars represent standard deviation. Animals treated with ZFP-R 89891 showed a decline in body weight starting around 12 days after test article administration, which may indicate potential toxicity of the 89891 ZFP-R.

[0023] **FIG. 5** is a panel of bar graphs demonstrating ZFP-R expression and human *PRNP* repression in a transgenic mouse model that is null for mouse PrP^C expression, and has been engineered to express the full-length human *PRNP* gene. Five ZFP-Rs were delivered to these mice using AAV-PHP.B intravenous delivery. Eight weeks after dosing, tissue was collected and RT-qPCR analysis was performed to assess the expression level of the human *PRNP* gene (top panel) and ZFP-R (bottom panel). The y-axis in each graph is normalized *PRNP* expression (top panel) or absolute ZFP-R mRNA expression (bottom panel). The expression level of the human *PRNP* gene was normalized to that of the house-keeping genes (*Atp5b* and *Eif4a2*) and scaled to the mean of the vehicle group. ZFP-Rs were dosed at 1E13 vg/kg ("-L" for Low dose) or 1E14 vg/kg ("-H" for High dose) and are indicated on the x-axis. The bars represent the mean of values from 5-8 mice. Error bars represent standard deviation. Dose-dependent expression of ZFP-R and repression of *PRNP* was observed. A one-way ANOVA analysis followed by Dunnett's multiple comparison test was used to determine statistical significance; P value <0.05 (*), <0.001 (**), <0.0001 (****).

[0024] **FIG. 6** is a panel of bar graphs showing the expression level of neuron and glia inflammatory markers from four different microdissected brain regions (sensory cortex, striatum, midbrain, cerebellum) 8 weeks after delivery of 5 ZFP-Rs using AAV-PHP.B at two dose levels in transgenic mice expressing the human *PRNP* gene. The y-axis in each graph is the expression *Iba1*, *Gfap, Rbfox3* and *Tubb3* (top to bottom panels) markers normalized to the expression of the house-keeping genes (*Atp5b* and *Eif4a2*) and scaled to the mean of the vehicle group. Each AAV.PHP.B ZFP-R was dosed at 1E13 vg/kg ("-L" for Low dose) or 1E14 vg/kg ("-H" for High dose) as indicated on the x-axis. The bars represent the mean of values from 5-8 mice. Error bars represent standard deviation. A one-way ANOVA analysis followed by Dunnett's multiple comparison test was used to determine statistical significance; P value <0.05 (*), <0.01 (**), <0.001 (***), <0.0001 (****).

[0025] **FIG. 7A** shows representative confocal images from animals treated at the 1E14 vg/kg (high) dose showing extensive human *PRNP* mRNA repression in neurons in the thalamus compared to the vehicle control at the single-cell level for all five ZFP-Rs tested. A multiplexed RNAscope *in situ* hybridization and immunohistochemistry method was used to detect ZFP-R (red) and *PRNP* (yellow) mRNA expression and immunohistochemistry for identifying neuronal cell types (purple).

[0026] **FIG. 7B** is a bar graph showing % human *PRNP* repression determined by a Spot total intensity parameter, which quantifies the RNAscope signal detected for human *PRNP* transcripts. The data are presented for neurons in the thalamus that were categorized as

having ZFP-R expression based on the RNAscope signal detected using a ZFP-R specific probe. ZFP-R and dosing groups are scaled to the vehicle control group. The upper horizontal line represents the normalized *PRNP* level in vehicle animals. N = 5-8. Downward arrows with percentages above each bar graph indicate the percent repression of human *PRNP* compared to the vehicle group.

[0027] **FIG. 8** is a panel of bar graphs demonstrating PrP protein repression in transgenic mice expressing human *PRNP* gene following treatment with ZFP-Rs delivered intravenously using AAV-PHP.B. Prion protein, PrP, was detected and quantified using an anti-PrP antibody-based assay in brain tissue (brainstem, top panel) and cerebrospinal fluid (CSF, bottom panel) 8 weeks after dosing. The y-axis in each graph is the PrP protein level scaled to the mean of the vehicle group. Each AAV.PHP.B ZFP-R was dosed at 1E13 vg/kg ("-L" for Low dose) or 1E14 vg/kg ("-H" for High dose) as indicated on the x-axis. The bars represent the mean of values from 5-8 mice and the error bars represent standard deviation. A dose-dependent reduction of brain and CSF PrP was observed for ZFP-R treated animals.

[0028] **FIG. 9** is a panel of graphs demonstrating the efficacy of AAV.PHP.B ZFP-R treatment in a mouse model of prion disease. The left panels show survival plots for mice treated with either one of two ZFP-Rs or control treatments at either 60 days post inoculation (dpi) (top left panel) or 122 dpi (bottom left panel). The x axis represents the number of days after the mice were inoculated with misfolded PrP^{Sc}. The y axis represents the percentage of surviving mice. The middle panels show the mean body weight measurements per group during the 500 day study. The dashed line at -20% indicates the relative body weight at which mice underwent preterminal humane euthanasia. Body weight averages are shown for groups treated with either one of two ZFP-Rs or control treatments at either 60 days post inoculation (dpi) (top middle panel) or 122 dpi (bottom middle panel). The right panels show the plasma levels of Neurofilament Light Chain (NfL) that received ZFP-R at 60 or 122 days. The average NfL levels are shown for groups treated with either one of two ZFP-Rs or control treatments at either 60 days post inoculation (dpi) (top right panel) or 122 dpi (bottom right panel). The data show that mice that received control treatments (AAV.PHP.B GFP or vehicle) reached terminal endpoint at 160±8 dpi (mean±sd) with a precipitous decline in body weight and increase in plasma NfL. In contrast, the majority of AAV ZFP-R treated mice (n=10/19) were alive at 1 year after inoculation with stable body weight and arrested NfL levels, indicating a beneficial effect of ZFP-R treatment at both early and late intervention points in the disease course. ZFP-R treated mice from both the 60 dpi and 122 dpi groups (total n=5) survived to the end of the study at 500 dpi.

[0029] **FIG. 10** is a panel of RT-qPCR expression data demonstrating *PRNP* gene repression in 35 different regions of the adult cynomolgus monkey brain. Brains tissue was analyzed 19 days after injection of an engineered blood-brain-barrier penetrant AAV capsid encoding a ZFP-R that targets the nonhuman primate *PRNP* gene. The x axis indicates the brain region from which punches were collected and analyzed. The y axis indicates the average expression level of the nonhuman primate *PRNP* gene normalized to that of the housekeeping genes (*Atp5b* and *Eif4a2*). For each punch, normalized *PRNP* expression was scaled to the mean of control treated animals. Each dot represents the scaled, normalized *PRNP* average across all the punches analyzed for one animal. The bar plot represents the average value for the 3 animals and the error bars represent standard deviation. The arrow pointing to a y-value of 1 indicates the average baseline level of prion obtained from prion expression in control-treated animals from the same study. *PRNP* gene repression ranged from 2-33% across the 35 brain regions analyzed.

DETAILED DESCRIPTION OF THE INVENTION

[0030] Disclosed are ZFP domains that target sites (*i.e.*, sequences) in or near the mammalian *PRNP* gene. A ZFP domain as described herein may be attached or fused to another functional molecule or domain (the functional molecule or domain can include a transcription repressor domain, epigenetic silencing domain, or other molecule or domain that reduces the transcription of a gene). The ZFP domains may be fused to a transcription factor to repress the transcription of the mammalian *PRNP* gene into RNA. The fusion proteins are called zinc finger protein repressors (ZFRs). These ZFRs comprise a zinc finger protein (ZFP) domain that binds specifically to a target region (*i.e.*, target site) in or near the *PRNP* gene and a transcription repressor domain that reduces the transcription of the gene. Reducing the level of PrP in neurons by introducing the ZFRs into the brain of a patient is expected to inhibit (*e.g.*, reduce or stop) the formation and spread of PrP^{Sc}, thereby treating prion disease (*e.g.*, alleviating symptoms, preventing onset or worsening of symptoms, and extending survival).

[0031] Our ZFR approach to *PRNP* inhibition has several advantages over the current approaches being tested by others. ZFRs can achieve higher levels of PrP repression than what has been reported for antisense oligonucleotides (ASOs). Further, ZFRs may need to be administered only once (by introducing to the patient a ZFR expression construct such as recombinant viruses, *e.g.*, recombinant AAV), while ASOs require repeated dosing. In addition, the ZFR approach only needs to engage the two alleles of the *PRNP* gene in the genome of each cell. By contrast, ASOs need to engage numerous copies of the *PRNP* mRNA

in each cell. In addition, with the use of recombinant viruses such as recombinant AAV, ZFRs can be delivered to any brain region of interest.

[0032] Disclosed are compositions comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding ZFP domains that target sites (*i.e.,* bind DNA sequences) in or near the human *PRNP* gene. The AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulates the expression construct to enable delivery of the construct across the blood brain barrier. A ZFP domain as described herein may be attached or fused to another functional molecule or domain. The ZFP domains disclosed herein may be fused to a transcription factor to repress transcription of the human *PRNP* gene into mRNA. The fusion proteins are called zinc finger protein-repressors (ZFRs) that target specifically the human *PRNP* gene and repress its transcription into RNA. These ZFRs comprise a zinc finger protein (ZFP) domain that binds specifically to a target region in or near the *PRNP* gene and a transcription repressor domain or epigenetic silencing domain that reduces the transcription of the gene. Reducing the level of PrP in neurons by introducing the ZFRs into the brain of a patient is expected to inhibit (*e.g.,* reduce or stop) transcription of the *PRNP* gene.

[0033] The AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct comprising a coding sequence for a fusion protein, the fusion protein comprising a *PRNP* targeting ZFP approach (See FIG. 1A) to PrP inhibition has several advantages over the current approaches being tested by others, which include administration of (i) antisense oligonucleotides (ASOs) that bind PrP mRNA and prevent its translation and (ii) immunotherapeutic anti-PrP antibodies. ZFRs may need to be administered only once (by introducing to the patient an expression construct encoding a ZFR encapsulated into an AAV capsid comprising SEQ ID NO: 1498, while ASOs require repeated dosing. In addition, the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct comprising a coding sequence for a fusion protein, the fusion protein comprising a *PRNP* targeting ZFP approach only needs to engage the two alleles of the *PRNP* gene in the genome of each cell. By contrast, ASOs need to engage numerous copies of the *PRNP* mRNA in each cell. Additionally, the distribution and tropism of ASOs is fixed, whereas the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an

amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct encoding the fusion protein, the fusion protein comprising a ZFP domain that binds to a target region of a human *PRNP* gene can be targeted to different cell types and brain regions by altering the promoter, serotype, and route of administration. As such, with the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct comprising a coding sequence for a fusion protein, the fusion protein comprising a *PRNP* targeting ZFP approach, all brain regions can be targeted which in turn translates to treatment for all tauopathy indications. Moreover, the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct comprising a coding sequence for a fusion protein, the fusion protein comprising a *PRNP* targeting ZFP approach can have cell type specificity, *i.e.,* be restricted to CNS cell types. Finally, the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct comprising a coding sequence for a fusion protein, the fusion protein comprising a *PRNP* targeting ZFP approach has rapid pharmacokinetics, *i.e.,* 90-100% single cell potency. In some embodiments, the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct comprising a coding sequence for a fusion protein, the fusion protein comprising a *PRNP* targeting ZFP approach uses an AAV capsid protein comprising SEQ ID. NO: 1498 and an expression construct comprising a fusion protein, the fusion protein comprising a ZFP domain the ZFP domain comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3. In some embodiments, the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct comprising a coding sequence for a fusion protein, the fusion protein comprising a *PRNP* targeting ZFP approach uses an AAV capsid protein comprising SEQ ID. NO: 1498 and an expression construct encoding a fusion protein comprising a ZFR shown in a single row of Tables 2 and 4.

[0034] Use of an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a *PRNP* targeted ZFP fusion protein is advantageous over the antibody approach because antibodies can only bind a subset of PrP protein species or conformations. This may not be sufficient for a robust therapeutic effect. In

contrast, ZFRs repress PrP expression at the DNA level and lead to lower levels of all forms of PrP, including different PrP conformers and post-translationally modified forms found across Tauopathies. ZFRs are therefore agnostic to the form of the toxic species, unlike antibodies. In addition, antibodies are thought to primarily act on extracellular PrP, whereas ZFRs can reduce total PrP levels inside the cell directly, thereby indirectly lowering extracellular PrP levels. Thus, use of an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 (preferably one of SEQ ID NOs: 333, 417, 442, or 673) and an expression construct encoding a *PRNP* targeted ZFP fusion protein (preferably the fusion protein comprises DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 and/or a ZFR shown in a single row of Tables 2 and 4) is expected to be more effective because PrP exerts its pathology intracellularly and the pathogenic species are unknown. Further, antibodies require repeated administration, typically into the periphery, which results in inefficient crossing of the blood-brain barrier, while ZFRs require only a one-time delivery of their expression constructs and can be administered via several routes, including directly to the brain parenchyma, into the CSF, or intravenously. In some embodiments, the composition for one-time delivery comprises and AAV capsid protein comprising SEQ ID NO: 1498 and an expression construct comprising a coding sequence for a fusion protein, the fusion protein comprising a ZFP domain the ZFP domain comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3. In some embodiments, the composition for one-time delivery comprises and AAV capsid protein comprising SEQ ID NO: 1498 and an expression construct comprising a coding sequence for a fusion protein, the fusion protein comprising a ZFR shown in a single row of Tables 2 and 4.

[0035] Disclosed is an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 (preferably one of SEQ ID NOs: 333, 417, 442, or 673) and an expression construct encoding a *PRNP* targeted ZFP fusion protein comprising a ZFP domain, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequence comprising SEQ ID NOS: 22-30, 31-39, 40-48, 49-57, 58-66, 67-73 ordered as shown in Table 1. Disclosed is an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 (preferably one of SEQ ID NOs: 333, 417, 442, or 673) and an expression construct encoding a *PRNP* targeted ZFP fusion protein comprising a ZFP domain, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequence capable of binding the

target sequence comprising, consisting, or consisting essentially of any one of SEQ ID NOS: 1, 2, 12, 16, 17, 18, 19, 20 or 21 as shown in Table 1. Disclosed is an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 (preferably one of SEQ ID NOs: 333, 417, 442, or 673) and an expression construct encoding a *PRNP* targeted ZFP fusion protein comprising a ZFP domain, the ZFP domain comprising, consisting, or consisting essentially of an amino acid sequence selected from SEQ ID NOs: 74-82 as shown in Table 2.

[0036] Disclosed is an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 (preferably one of SEQ ID NOs: 333, 417, 442, or 673) and an expression construct encoding a *PRNP* targeted ZFP fusion protein comprising a ZFP domain, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequence comprising SEQ ID NOS: 112-123, 124-135, 136-147, 148-159, 160-171, 172-181 ordered as shown in Table 3. Disclosed is an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 (preferably one of SEQ ID NOs: 333, 417, 442, or 673) and an expression construct encoding a *PRNP* targeted ZFP fusion protein comprising a ZFP domain, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequence capable of binding the target sequence comprising, consisting, or consisting essentially of any one of SEQ ID NOS: 83-111 as shown in Table 3. Disclosed is an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 (preferably one of SEQ ID NOs: 333, 417, 442, or 673) and an expression construct encoding a *PRNP* targeted ZFP fusion protein comprising a ZFP domain, the ZFP domain comprising, consisting, or consisting essentially of an amino acid sequence selected from SEQ ID NOs: 183-194 as shown in Table 4.

[0037] Our approach to prion disease treatment is expected to be safe. About 1:18,000 humans are estimated to be heterozygous for loss-of-function *PRNP* mutation and yet there are no discernible deleterious effects in these individuals (Minikel et al., 2020).

[0038] Zinc finger protein transcription factors for repressing PrP expression are disclosed in International Patent Publication WO 2021/067864, the entirety of which is incorporated by reference herein (PCT Pat. App. No. PCT/US2020/054140). Specifically, sections related to sequence listings, Targets of the ZFP Domains, ZFP Domains, Zinc Finger Protein Transcription Factors, Zinc Finger Repression Domains, Peptide Linkers, Expression

of the ZFRs and Pharmaceutical Applications are incorporated by reference herein in their entirety.

[0039] Fitness maturation of engineered AVV capsid stac-102 is disclosed in International Patent Publication WO WO2024238579, the entirety of which is incorporated by reference herein (PCT Pat. App. No. PCT/US2017/064181). Engineered blood brain barrier penetrant AAV capsids are disclosed in International Patent Publication WO 2024/238684, the entirety of which is incorporated by reference herein (PCT Pat. App. No. PCT/US2024/029507). Specifically, sections related to Libraries of AAV Capsid Proteins, Engineered AAV Capsid Proteins, Development of engineered AAV capsid proteins, Gene editing system, Engineered AAV capsid proteins within a cell, Engineered AAV capsid proteins delivered to a target cell, Methods of detecting engineered AAV capsid proteins, Capsid proteins, CNS (Central Nervous System)- Targeting Molecules (Targeting Peptides), AAV particles, Delivery of AAV Particles, Insertion of Targeting Peptides into Capsid Proteins, and Pharmaceutical Compositions and Dosage Forms are incorporated by reference herein in their entirety.

I. Targets of the ZFP Domains

[0040] Zinc finger protein transcription factors for repressing PrP expression is disclosed in International Patent Publication WO 2021/067864, the entirety of which is incorporated by reference herein and specifically incorporated by reference in its entirety is the section related to Targets of the ZFP Domains.

[0041] The ZFP domains of the fusion proteins bind specifically to a target region in or near the mammalian (*e.g.*, human, non-human primate,) *PRNP* gene. The DNA-binding ZFP domain of the ZFRs directs the fusion proteins to a target region of the *PRNP* gene and brings the transcription repressor domain of the fusion proteins to the target region. The repressor domain then represses the *PRNP* gene's transcription by RNA polymerase. The target region can be any suitable site in the *PRNP* gene that allows repression of gene expression. By way of example, the target region includes, or is adjacent to (either downstream or upstream of) a *PRNP* transcription start site (TSS), or a *PRNP* transcription regulatory element (*e.g.*, promoter, enhancer, RNA polymerase pause site, exon/intron boundary, and the like). For example, the target region may be within about 500-1,000 bp upstream and/or downstream of the TSS.

[0042] In some embodiments, the genomic target region is at least 8 bps in length. For example, the target region may be 8 bps to 40 bps in length, such as 12, 15, 18, 21, 24, 27,

30, 33, or 36 bps in length. The targeted sequence may be on the sense strand of the gene, or the antisense strand of the gene. To ensure targeting accuracy and to reduce off-target binding or activity by the ZFRs, the sequence of the selected *PRNP* target region preferably has less than 75% homology (*e.g.*, less than 70%, less than 65%, less than 60%, or less than 50%) to sequences in other genes. In certain embodiments, the target region of the ZFRs is 15 -18 bps in length and resides within about 500-1,000 bps of the TSS. Examples of target regions in the human *PRNP* gene are shown.

[0043] In some embodiments, the engineered ZFPs bind to a target site (*i.e.*, Binding Sequence or Target Sequence) as shown in a single row of Tables 1 and 3 preferably with no or little detectable off-target binding or activity.

[0044] Other criteria for further evaluating target segments include the prior availability of ZFPs binding to such segments or related segments, ease of designing new ZFPs to bind a given target segment, and predicted off-target binding assessment.

Table 1.

ZFP ID	Target Sequence (capital letters)	DNA-Recognition Helix Amino Acid Sequence Within Each Finger											
		F1	L1	F2	L2	F3	L3	F4	L4	F5	L5	F6	L6
ZFC81613	caCTTGCATCAGTTGATACCgcctgcgg (SEQ ID NO: 1)	YPKDLAR(SEQ ID NO: 22)	0	TSGNLTR(SEQ ID NO: 31)	0c	TSGSLSR(SEQ ID NO: 40)	0	QSADRTK(SEQ ID NO: 49)	0c	QSGDLTR(SEQ ID NO: 58)	0	YRWLRNN(SEQ ID NO: 67)	C1
ZFC81747	gcCTCGGTCGTGAGgAGAGGAgaagctc (SEQ ID NO: 2)	QSGHLAR(SEQ ID NO: 23)	0	QLTHLNS(SEQ ID NO: 32)	1c	RSDNLAR(SEQ ID NO: 41)	0	MRHHLDD(SEQ ID NO: 50)	0c	LRHHLTR(SEQ ID NO: 59)	0	DRSYRNT(SEQ ID NO: 68)	C1
ZFC89324	ttTACCTGcCTCGGTCGTGAGgagaggga (SEQ ID NO: 12)	RSDNLAR(SEQ ID NO: 24)	0	MRHHLDD(SEQ ID NO: 33)	0c	LRHHLTR(SEQ ID NO: 42)	0	DRSYRNT(SEQ ID NO: 51)	1c	RSDVLSE(SEQ ID NO: 60)	0	DRSNRIK(SEQ ID NO: 69)	C1
ZFC89322	gtCGTGAGgAGAGGAG	QSSDLSR(SE	0	QSGNLAR(SE	0c	QSGHLAR(SEQ ID	0	QLTHLNS(SE	1c	RSDNLAR(SE	0	MRHHLDD(SE	C1

	AAGCTcgcg gcg (SEQ ID NO: 16)	Q ID NO: 25)		Q ID NO: 34)		NO: 43)		Q ID NO: 52)		Q ID NO: 61)		Q ID NO: 70)	
ZFC 91973	ggGCGCCT gGGGGCCG CGGGGctcg cgc (SEQ ID NO: 17)	RSA HLS R(SEQ ID NO: 26)	0	QSSD LRR(SEQ ID NO: 35)	0 c	DRSD LSR(S EQ ID NO: 44)	0	RST HLV R(SEQ ID NO: 53)	1 c	QQS DLR V(SEQ ID NO: 62)	0	RSA DLS R(SEQ ID NO: 71)	C 1
ZFC 89302	cgATGGCA cCCGCAGG CGGTAtcaac tg (SEQ ID NO: 18)	QSG ALA R(SEQ ID NO: 27)	0	RSDD LTR(SEQ ID NO: 36)	0 c	RSDN LSA(S EQ ID NO: 45)	0	RNN DRK T(SEQ ID NO: 54)	1 c	QSG DLTR (SEQ ID NO: 63)	0	RKD PLK E(SEQ ID NO: 72)	C 1
ZFC 89427	tcCGAGGG GCGGCCA ACGGGcggg gagc (SEQ ID NO: 19)	RSD HLS R(SEQ ID NO: 28)	0	DISN RNA(SEQ ID NO: 37)	0 c	DRST RTK(S EQ ID NO: 46)	0	RSD DRK K(SEQ ID NO: 55)	0 c	RSD HLSR (SEQ ID NO: 64)	0	QKC HLR S(SEQ ID NO: 73)	C 1
ZFC 89352	cgCGTGAC GCGTCTCG Ggcctgcccgg c (SEQ ID NO: 20)	RSD HLS E(SEQ ID NO: 29)	0	RKSD RIK(S EQ ID NO: 38)	0 c	RSAD LTR(S EQ ID NO: 47)	0	DRS NLT R(SEQ ID NO: 56)	0 a	HKQ HRD A(SEQ ID NO: 65)			C 1
ZFC 89347	cgCGGGCG CGTGACGC Gtctcgggcctg (SEQ ID NO: 21)	RSA DLT R(SEQ ID NO: 30)	0	DRSN LTR(SEQ ID NO: 39)	0 c	RKQT RTT(S EQ ID NO: 48)	0	RRS DLK R(SEQ ID NO: 57)	0 a	RNG HLL D(SEQ ID NO: 66)			C 1

Table 1 shows exemplary ZFPs that target human *PRNP*. Shown in capital letters are the genomic target sequences (*i.e.*, bound sequences) of the DNA-binding recognition helix sequences that are shown in a single row for each five or six finger ZFP shown (*i.e.*, F1-F5, F1-F6, or F1 and F3-F6). Illustrative peptide linker sequences as shown in Table 5 between zinc fingers and between the ZFP domain and the repressor domain for each ZFP are also shown (*i.e.*, L1, L2, L3, L4, L5, or L6).

II. ZFP Domains

[0045] Zinc finger protein transcription factors for repressing PrP expression is disclosed in International Patent Publication WO 2021/067864, the entirety of which is incorporated by reference herein and specifically incorporated by reference in its entirety is the section related to Zinc Finger Protein Domains.

[0046] A "zinc finger protein" or "ZFP" refers to a protein having a DNA-binding domain that is stabilized by zinc. ZFPs bind to DNA in a sequence-specific manner. The individual DNA-binding unit of a ZFP is referred to as a zinc "finger". Each finger contains a DNA-binding "recognition helix" that is typically comprised of seven amino acid residues and determines DNA binding specificity. A ZFP domain has at least one finger, each finger binds from two to four base pairs of DNA, typically three or four base pairs of DNA. Each zinc finger typically comprises approximately 30 amino acids and chelates zinc. An engineered ZFP can have a novel binding specificity, compared to a naturally-occurring ZFP. Engineering methods include, but are not limited to, rational design and various types of selection. Rational design includes, for example, using databases comprising triplet (or quadruplet) nucleotide sequences and individual zinc finger amino acid sequences, in which each triplet or quadruplet nucleotide sequence is associated with one or more amino acid sequences of zinc fingers that bind the particular triplet or quadruplet sequence. *See, e.g.,* ZFP design methods described in detail in U.S. Pats. 5,789,538 (herein incorporated by reference in its entirety); 5,925,523 (herein incorporated by reference in its entirety); 6,007,988 (herein incorporated by reference in its entirety); 6,013,453 (herein incorporated by reference in its entirety); 6,140,081 (herein incorporated by reference in its entirety); 6,200,759 (herein incorporated by reference in its entirety); 6,453,242 (herein incorporated by reference in its entirety); 6,534,261 (herein incorporated by reference in its entirety); 6,979,539 (herein incorporated by reference in its entirety); and 8,586,526 ((herein incorporated by reference in its entirety); and International Patent Publications WO 95/19431 (herein incorporated by reference in its entirety); WO 96/06166 (herein incorporated by reference in its entirety); WO 98/53057 (herein incorporated by reference in its entirety); WO 98/53058 (herein incorporated by reference in its entirety); WO 98/53059 (herein incorporated by reference in its entirety); WO 98/53060 (herein incorporated by reference in its entirety); WO 98/54311 (herein incorporated by reference in its entirety); WO 00/27878 (herein incorporated by reference in its entirety); WO 01/60970 (herein incorporated by reference in its entirety); WO 01/88197 (herein incorporated by reference in its entirety); WO 02/016536 (herein incorporated by reference in its entirety); WO 02/099084 (herein incorporated by reference in its entirety); and WO 03/016496 (herein incorporated by reference in its entirety). A ZFP domain as described herein may be attached or fused to another molecule, for example, a protein. Such ZFP-fusions may comprise a domain that enables gene activation (*e.g.*, activation domain), gene repression (*e.g.*, repression domain), ligand binding (*e.g.*, ligand-binding domain), high-throughput screening (*e.g.*, ligand-binding domain), localized hypermutation (*e.g.*, activation-induced

cytidine deaminase domain), chromatin modification (*e.g.*, histone deacetylase domain), recombination (*e.g.*, recombinase domain), targeted integration (*e.g.*, integrase domain), DNA modification (*e.g.*, DNA methyl-transferase domain), base editing (*e.g.*, base editor domain), or targeted DNA cleavage (*e.g.*, nuclease domain). Examples of engineered ZFP domains are shown in Tables 1 and 3. Polypeptide sequences of the ZFRs in Table 1, are shown in Table 2. Polypeptide sequences of the ZFRs in Table 3, are shown in Table 4.

Table 2.

ZFP ID	Amino Acid sequence (**helix**, ZF linker, & interdomain linker)
ZFC81613	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**YPKDLAR**HIRTHTGEKPFACDICGRKFA**TSGNLTR**HTKIHTGSQKPFQCRICMRNFS**TSGSLSR**HIRTHTGEKPFACDICGRKFA**QSADRTK**HTKIHTGSQKPFQCRICMRNFS**QSGDLTR**HIRTHTGEKPFACDICGRKFA**YRWLRNN**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 74)
ZFC81747	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**QSGHLAR**HIRTHTGEKPFACDICGRKFA**QLTHLNS**HTKIHTHPRAPIPKPFQCRICMRNFS**RSDNLAR**HIRTHTGEKPFACDICGRKFA**MRHHLDD**HTKIHTGSQKPFQCRICMRNFS**LRHHLTR**HIRTHTGEKPFACDICGRKFA**DRSYRNT**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 75)
ZFC89324	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**RSDNLAR**HIRTHTGEKPFACDICGRKFA**MRHHLDD**HTKIHTGSQKPFQCRICMRNFS**LRHHLTR**HIRTHTGEKPFACDICGRKFA**DRSYRNT**HTKIHTHPRAPIPKPFQCRICMRNFS**RSDVLSE**HIRTHTGEKPFACDICGRKFA**DRSNRIK**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 76)
ZFC89322	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**QSSDLSR**HIRTHTGEKPFACDICGRKFA**QSGNLAR**HTKIHTGSQKPFQCRICMRNFS**QSGHLAR**HIRTHTGEKPFACDICGRKFA**QLTHLNS**HTKIHTHPRAPIPKPFQCRICMRNFS**RSDNLAR**HIRTHTGEKPFACDICGRKFA**MRHHLDD**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 77)
ZFC91973	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**RSAHLSR**HIRTHTGEKPFACDICGRKFA**QSSDLRR**HTKIHTGSQKPFQCRICMRNFS**DRSDLSR**HIRTHTGEKPFACDICGRKFA**RSTHLVR**HTKIHTHPRAPIPKPFQCRICMRNFS**QQSDLRV**HIRTHTGEKPFACDICGRKFA**RSADLSR**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 78)

ZFP ID	Amino Acid sequence (**helix**, ZF linker, & interdomain linker)
ZFC89302	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**QSGALAR**HIRTHTGEKPFACDICGRKFA**RSDDLTR**HTKIHTGSQKPFQCRICMRNFS**RSDNLSA**HIRTHTGEKPFACDICGRKFA**RNNDRKT**HTKIHTHPRAPIPKPFQCRICMRNFS**QSGDLTR**HIRTHTGEKPFACDICGRKFA**RKDPLKE**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 79)
ZFC89427	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**RSDHLSR**HIRTHTGEKPFACDICGRKFA**DISNRNA**HTKIHTGSQKPFQCRICMRNFS**DRSTRTK**HIRTHTGEKPFACDICGRKFA**RSDDRKK**HTKIHTGSQKPFQCRICMRNFS**RSDHLSR**HIRTHTGEKPFACDICGRKFA**QKCHLRS**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 80)
ZFC89352	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**RSDHLSE**HIRTHTGEKPFACDICGRKFA**RKSDRIK**HTKIHTGSQKPFQCRICMRNFS**RSADLTR**HIRTHTGEKPFACDICGRKFA**DRSNLTR**HTKIHTGEKPFQCRICMRKFA**HKQHRDA**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 81)
ZFC89347	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**RSADLTR**HIRTHTGEKPFACDICGRKFA**DRSNLTR**HTKIHTGSQKPFQCRICMRNFS**RKQTRT**THIRTHTGEKPFACDICGRKFA**RRSDLKR**HTKIHTGEKPFQCRICMRKFA**RNGHLLD**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 82)

Table 2 shows illustrative polypeptide sequences for the ZFRs shown in Table 1. DNA-binding recognition helix sequences are in boldface. Zinc finger linkers are underlined, whereas interdomain linkers are double underlined.

[0047] The ZFP domain of the engineered ZFP fusion proteins may include at least one (*e.g.*, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or more) zinc finger(s). A ZFP domain having one finger typically recognizes a target site that includes 3 or 4 nucleotides. A ZFP domain having two fingers typically recognizes a target site that includes 6 or 8 nucleotides. A ZFP domain having three fingers typically recognizes a target site that includes 9 or 12 nucleotides. A ZFP domain having four fingers typically recognizes a target site that includes 12 to 15 nucleotides. A ZFP domain having five fingers typically recognizes a target site that includes 15 to 18 nucleotides. A ZFP domain having six fingers can recognize target sites that include 18 to 21 nucleotides.

[0048] In some embodiments, the engineered ZFPs comprise a DNA-binding recognition helix sequence shown in Tables 1 and 3. For example, an engineered ZFP may comprise the sequence of F1, F2, F3, F4, F5, or F6 as shown in Tables 1 and 3.

[0049] In some embodiments, the engineered ZFPs comprise two adjacent DNA-binding recognition helix sequences shown in a single row of Tables 1 and 3. For example, an engineered ZFP may comprise the sequences of F1-F2, F2-F3, F3-F4, F4-F5, or F5-F6 as shown in a single row of Tables 1 and 3.

[0050] In some embodiments, the engineered ZFPs comprise the DNA-binding recognition helix sequences shown in a single row of Tables 1 and 3. For example, an engineered ZFP may comprise the sequences of F1, F2, F3, F4, F5, and F6 (*e.g.*, F1-F6) ordered as shown in a single row of Tables 1 and 3.

[0051] The target specificity of the ZFP domain may be improved by mutations to the ZFP backbone sequence as described in, *e.g.*, U.S. Pat. Pub. 2018/0087072. The mutations include those made to residues in the ZFP backbone that can interact non-specifically with phosphates on the DNA backbone but are not involved in nucleotide target specificity (*see, e.g.,* Miller et al., *Nat Biotechnol.* (2019) 37(8):945-52). In some embodiments, these mutations comprise mutating a cationic amino acid residue to a neutral or anionic amino acid residue. In some embodiments, these mutations comprise mutating a polar amino acid residue to a neutral or non-polar amino acid residue. In further embodiments, mutations are made at positions (-5), (-9) and/or (-14) relative to the DNA binding helix. In some embodiments, a zinc finger may comprise one or more mutations at positions (-5), (-9) and/or (-14). In further embodiments, one or more zinc fingers in a multi-finger ZFP domain may comprise mutations at positions (-5), (-9) and/or (-14). In some embodiments, the amino acids at positions (-5), (-9) and/or (-14) (*e.g.*, an arginine (R) or lysine (K)) are mutated to an alanine (A), leucine (L), serine (S), aspartate (N), glutamate (E), tyrosine (Y), and/or glutamine (Q). Examples of engineered ZFPs with backbone mutations are shown in Table 3. The symbol "^" in Table 3 indicates that arginine (R) residue at the 4th position upstream of the 1st amino acid in the indicated recognition helix is changed to glutamine (Q), which is shown in bold in Table 4. In each recognition helix sequence, the positions of the seven DNA-binding amino acids are numbered -1, +1, +2, +3, +4, +5, and +6. Thus, the position for the R-to-Q substitution is numbered as (-5).

Table 3.

ZFP ID	Target Sequence (capital letters)	DNA-Recognition Helix Amino Acid Sequence Within Each Finger											
		F1	L1	F2	L2	F3	L3	F4	L4	F5	L5	F6	L6
ZFC81638	ttTAAGTTAAAGGGtGCAGCGctgccaa (SEQ ID NO: 83)	^RSADLTR (SEQ ID NO: 112)	0	QSGDLTR(SEQ ID NO: 124)	1c	^RSDHLSR (SEQ ID NO: 136)	0	DRSNRKT(SEQ ID NO: 148)	0c	^TSGSLSR(SEQ ID NO: 160)	0	QNANLMK(SEQ ID NO: 172)	C1
ZFC89891	caCTTGCATCAGTTGATACCgcctgcgg (SEQ ID NO: 84)	YPKDLAR(SEQ ID NO: 113)	0	TSGNLTR(SEQ ID NO: 125)	0c	^TSGSLSR(SEQ ID NO: 137)	0	QSADRTK (SEQ ID NO: 149)	0c	^QSGDLTR(SEQ ID NO: 161)	0	YRWLRNN(SEQ ID NO: 173)	C1
ZFC89920	ttTAAGTTAAAGGGtGCAGCGctgccaa (SEQ ID NO: 85)	^RSADLTR (SEQ ID NO: 114)	0	QSGDLTR(SEQ ID NO: 126)	1c	^RSDHLSR (SEQ ID NO: 138)	0	DRSNRKT(SEQ ID NO: 150)	0c	^TSGSLSR(SEQ ID NO: 162)	0	^QNANLMK(SEQ ID NO: 174)	C1
ZFC89943	gcCTCGGTCGTGAGgAGAGGAgaagctc (SEQ ID NO: 86)	^QSGHLAR(SEQ ID NO: 115)	0	^QLTHLNS(SEQ ID NO: 127)	1c	^RSDNLAR(SEQ ID NO: 139)	0	MRHHLDD(SEQ ID NO: 151)	0c	^LRHHLTR(SEQ ID NO: 163)	0	^DRSYRNT (SEQ ID NO:	C1

ZFP ID	Target Sequence (capital letters)	DNA-Recognition Helix Amino Acid Sequence Within Each Finger											
		F1	L1	F2	L2	F3	L3	F4	L4	F5	L5	F6	L6
												174)	
ZFC91806	cgCGTGACCGCGTCTCGGgcctgcccggc (SEQ ID NO: 87)	^RSDHLSE(SEQ ID NO: 116)	0	RKSDRIK(SEQ ID NO: 128)	0c	RSADLTR(SEQ ID NO: 140)	0	DRSNLTR(SEQ ID NO: 152)	0a	HKQHRDA(SEQ ID NO: 164)			C1
ZFC91852	ttTACCTGcCTCGGTCGTGAGgagagga (SEQ ID NO: 88)	^RSDNLAR(SEQ ID NO: 117)	0	MRHHLDD(SEQ ID NO: 129)	0c	LRHHLTR(SEQ ID NO: 141)	0	DRSYRNT(SEQ ID NO: 153)	1c	RSDVLSE(SEQ ID NO: 165)	0	DRSNRIK(SEQ ID NO: 175)	C1
ZFC91853	ttTACCTGcCTCGGTCGTGAGgagagga (SEQ ID NO: 89)	^RSDNLAR(SEQ ID NO: 118)	0	MRHHLDD(SEQ ID NO: 130)	0c	^LRHHLTR(SEQ ID NO: 142)	0	DRSYRNT(SEQ ID NO: 154)	1c	RSDVLSE(SEQ ID NO: 166)	0	DRSNRIK(SEQ ID NO: 176)	C1
ZFC91870	gtCGTGAGgAGAGGAGAAGCTcgcggcg (SEQ ID NO: 90)	^QSSDLSR(SEQ ID NO: 119)	0	^QSGNLAR(SEQ ID NO: 131)	0c	^QSGHLAR(SEQ ID NO: 143)	0	^QLTHLNS(SEQ ID NO: 155)	1c	^RSDNLAR(SEQ ID NO: 167)	0	MRHHLDD(SEQ ID NO: 177)	C1
ZFC91888	agGGAGCTtTCCTA	^QSGN	0	HNSSLK	0c	^QSAN	0	DSSDR	1c	^QSSDLS	0	QSGH	C1

ZFP ID	Target Sequence (capital letters)	DNA-Recognition Helix Amino Acid Sequence Within Each Finger										
		F1	L1	F2	L2	F3	L3	F4	L4	F5	L5	F6	L6
	ACCTGAAaagcgaa (SEQ ID NO: 91)	LAR(SEQ ID NO: 120)		D(SEQ ID NO: 132)		RTK(SEQ ID NO: 144)		KK(SEQ ID NO: 156)		R(SEQ ID NO: 168)		LSR(SEQ ID NO: 178)	
ZFC91975	ggGCGCCTgGGGGCCGCGGGGctcgcgc (SEQ ID NO: 92)	^RSAHLSR (SEQ ID NO: 121)	0	QSSDLRR(SEQ ID NO: 133)	0c	^DRSDLSR(SEQ ID NO: 145)	0	RSTHLVR(SEQ ID NO: 157)	1c	QQSDLRV(SEQ ID NO: 169)	0	RSADLSR(SEQ ID NO: 179)	C1
ZFC91999	cgATGGCAcCCGCAGGCGGTAtcaactg (SEQ ID NO: 110)	^QSGALAR(SEQ ID NO: 122)	0	^RSDDLTR(SEQ ID NO: 134)	0c	^RSDNLSA(SEQ ID NO: 146)	0	^RNNDRKT(SEQ ID NO: 158)	1c	^QSGDLTR(SEQ ID NO: 170)	0	^RKDPLKE(SEQ ID NO: 180)	C1
ZFC92071	tcCGAGGGGCGGCCAACGGGcgggggagc (SEQ ID NO: 111)	^RSDHLSR (SEQ ID NO: 123)	0	DISNRNA(SEQ ID NO: 135)	0c	^DRSTRTK(SEQ ID NO: 147)	0	RSDDRKK(SEQ ID NO: 159)	0c	RSDHLSR(SEQ ID NO: 171)	0	QKCHLRS(SEQ ID NO: 181)	C1

Table 3 shows exemplary R→Q (Arg -> Gln) variants of representative ZFRs targeting the human *PRNP* gene. Shown in capital letters are the genomic target sequences (*i.e.*, bound sequences) of the DNA-binding recognition helix sequences that are shown in a single row for each five or six finger ZFP shown (*i.e.*, F1-F5 or F1-F6). Table 3 also indicates illustrative peptide linker sequences as shown in Table 5 between zinc fingers and between the ZFP domain and the repressor domain for each ZFP shown (*i.e.*, L1, L2, L3, L4, L5, or L6). The

symbol "^" indicates that the arginine (R) residue at the 4th position upstream of the 1st amino acid in the indicated finger is changed to glutamine (Q).

Table 4.

ZFP ID	Amino Acid sequence (**helix**, *PCV*, ZF linker, & interdomain linker)
ZFC81638	MAPKKKRKVGVPAAMAERPFQCRICM*Q*NFS**RSADLTR**HIRTHTGEKPFACDICGRKFA**QSGDLTR**HTKIHTHPRAPIPKPFQCRICM*Q*NFS**RSDHLSR**HIRTHTGEKPFACDICGRKFA**DRSNRKT**HTKIHTGSQKPFQCRICM*Q*NFS**TSGSLSR**HIRTHTGEKPFACDICGRKFA**QNANLMK**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 183)
ZFC89891	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**YPKDLAR**HIRTHTGEKPFACDICGRKFA**TSGNLTR**HTKIHTGSQKPFQCRICM*Q*NFS**TSGSLSR**HIRTHTGEKPFACDICGRKFA**QSADRTK**HTKIHTGSQKPFQCRICM*Q*NFS**QSGDLTR**HIRTHTGEKPFACDICGRKFA**YRWLRNN**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 184)
ZFC89920	MAPKKKRKVGVPAAMAERPFQCRICM*Q*NFS**RSADLTR**HIRTHTGEKPFACDICGRKFA**QSGDLTR**HTKIHTHPRAPIPKPFQCRICM*Q*NFS**RSDHLSR**HIRTHTGEKPFACDICGRKFA**DRSNRKT**HTKIHTGSQKPFQCRICM*Q*NFS**TSGSLSR**HIRTHTGEKPFACDICG*Q*KFA**QNANLMK**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 185)
ZFC89943	MAPKKKRKVGVPAAMAERPFQCRICM*Q*NFS**QSGHLAR**HIRTHTGEKPFACDICG*Q*KFA**QLTHLNS**HTKIHTHPRAPIPKPFQCRICM*Q*NFS**RSDNLAR**HIRTHTGEKPFACDICGRKFA**MRHHLDD**HTKIHTGSQKPFQCRICM*Q*NFS**LRHHLTR**HIRTHTGEKPFACDICG*Q*KFA**DRSYRNT**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 186)
ZFC91806	MAPKKKRKVGVPAAMAERPFQCRICM*Q*NFS**RSDHLSE**HIRTHTGEKPFACDICGRKFA**RKSDRIK**HTKIHTGSQKPFQCRICMRNFS**RSADLTR**HIRTHTGEKPFACDICGRKFA**DRSNLTR**HTKIHTGEKPFQCRICMRKFA**HKQHRDA**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 187)
ZFC91852	MAPKKKRKVGVPAAMAERPFQCRICM*Q*NFS**RSDNLAR**HIRTHTGEKPFACDICGRKFA**MRHHLDD**HTKIHTGSQKPFQCRICMRNFS**LRHHLTR**HIRTHTGEKPFACDICGRKFA**DRSYRNT**HTKIHTHPRAPIPKPFQCRICMRNFS**RSDVLSE**HIRTHTGEKPFACDICGRKFA**DRSNRIK**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 188)

ZFP ID	Amino Acid sequence (**helix**, *PCV*, ZF linker, & interdomain linker)
ZFC91853	MAPKKKRKVGVPAAMAERPFQCRICM*Q*NFS**RSDNLAR**HIRTHTGEKPFACDICGRKFA**MRHHLDD**HTKIHTGSQKPFQCRICM*Q*NFS**LRHHLTR**HIRTHTGEKPFACDICGRKFA**DRSYRNT**HTKIHTHPRAPIPKPFQCRICMRNFS**RSDVLSE**HIRTHTGEKPFACDICGRKFA**DRSNRIK**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 189)
ZFC91870	MAPKKKRKVGVPAAMAERPFQCRICM*Q*NFS**QSSDLSR**HIRTHTGEKPFACDICG*Q*KFA**QSGNLAR**HTKIHTGSQKPFQCRICM*Q*NFS**QSGHLAR**HIRTHTGEKPFACDICG*Q*KFA**QLTHLNS**HTKIHTHPRAPIPKPFQCRICM*Q*NFS**RSDNLAR**HIRTHTGEKPFACDICGRKFA**MRHHLDD**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 190)
ZFC91888	MAPKKKRKVGVPAAMAERPFQCRICM*Q*NFS**QSGNLAR**HIRTHTGEKPFACDICGRKFA**HNSSLKD**HTKIHTGSQKPFQCRICM*Q*NFS**QSANRTK**HIRTHTGEKPFACDICGRKFA**DSSDRKK**HTKIHTHPRAPIPKPFQCRICM*Q*NFS**QSSDLSR**HIRTHTGEKPFACDICGRKFA**QSGHLSR**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 191)
ZFC91975	MAPKKKRKVGVPAAMAERPFQCRICM*Q*NFS**RSAHLSR**HIRTHTGEKPFACDICGRKFA**QSSDLRR**HTKIHTGSQKPFQCRICM*Q*NFS**DRSDLSR**HIRTHTGEKPFACDICGRKFA**RSTHLVR**HTKIHTHPRAPIPKPFQCRICMRNFS**QQSDLRV**HIRTHTGEKPFACDICGRKFA**RSADLSR**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 192)
ZFC91999	MAPKKKRKVGVPAAMAERPFQCRICM*Q*NFS**QSGALAR**HIRTHTGEKPFACDICG*Q*KFA**RSDDLTR**HTKIHTGSQKPFQCRICM*Q*NFS**RSDNLSA**HIRTHTGEKPFACDICG*Q*KFA**RNNDRKT**HTKIHTHPRAPIPKPFQCRICM*Q*NFS**QSGDLTR**HIRTHTGEKPFACDICG*Q*KFA**RKDPLKE**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 193)
ZFC92071	MAPKKKRKVGVPAAMAERPFQCRICM*Q*NFS**RSDHLSR**HIRTHTGEKPFACDICGRKFA**DISNRNA**HTKIHTGSQKPFQCRICM*Q*NFS**DRSTRTK**HIRTHTGEKPFACDICGRKFA**RSDDRKK**HTKIHTGSQKPFQCRICMRNFS**RSDHLSR**HIRTHTGEKPFACDICGRKFA**QKCHLRS**HTKIH***LRQKDAARGS***GGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSVRS(SEQ ID NO: 194)

Table 4 shows illustrative full protein sequences for R→Q (Arg -> Gln) variants of the ZFRs shown in Table 3. DNA-binding recognition helix sequences are in boldface. Zinc finger linkers are underlined, whereas interdomain linkers are double underlined.

[0052] In some embodiments, the engineered ZFPs comprise a DNA-binding recognition helix sequence and associated backbone mutation as shown in Table 3. In some embodiments, the engineered ZFPs comprise the DNA-binding recognition helix sequences and associated backbone mutations as shown in a single row of Table 3.

[0053] In some embodiments, an engineered ZFP described herein comprises the recognition helix and backbone portions of a sequence shown in a single row of Table 4. In some embodiments, an engineered ZFP described herein comprises the recognition helix and backbone portions of a sequence shown in a single row of Table 4 as the sequence would appear following post-translational modification. For example, post-translational modification may remove the initiator methionine residue from a sequence as shown in a single row of Table 4.

[0054] In some embodiments, the ZFRs comprise one or more zinc finger domains. The domains may be linked together via an extendable flexible linker such that, for example, one domain comprises one or more (*e.g.*, 4, 5, or 6) zinc fingers and another domain comprises additional one or more (*e.g.*, 4, 5, or 6) zinc fingers. In some embodiments, the linker is a standard inter-finger linker such that the finger array comprises one DNA binding domain comprising 8, 9, 10, 11 or 12 or more fingers. In other embodiments, the linker is an atypical linker such as a flexible linker. For example, two ZFP domains may be linked to a transcription repressor TF in the configuration (from N terminus to C terminus) ZFP-ZFP-TF, TF-ZFP-ZFP, ZFP-TF-ZFP, or ZFP-TF-ZFP-TF (two ZFP-TF fusion proteins are fused together via a linker).

[0055] In some embodiments, the ZFRs are "two-handed," *i.e.*, they contain two zinc finger clusters (two ZFP domains) separated by intervening amino acids so that the two ZFP domains bind to two discontinuous target sites. An example of a two-handed type of zinc finger binding protein is SIP1, where a cluster of four zinc fingers is located at the amino terminus of the protein and a cluster of three fingers is located at the carboxyl terminus (*see* Remacle et al., *EMBO J.* (1999) 18(18):5073-84). Each cluster of zinc fingers in these proteins is able to bind to a unique target sequence and the spacing between the two target sequences can comprise many nucleotides.

[0056] Alternatively, the DNA-binding domain may be derived from a nuclease. For example, the recognition sequences of homing endonucleases and meganucleases such as I-*Sce*I, I-*Ceu*I, PI-*Psp*I, PI-*Sce*, I-*Sce*IV, I-*Csm*I, I-*Pan*I, I-*Sce*II, I-*Ppo*I, I-*Sce*III, I-*Cre*I, I-*Tev*I, I-*Tev*II and I-*Tev*III are known. *See also* U.S. Pats 5,420,032 and 6,833,252; Belfort et al., *Nucleic Acids Res.* (1997) 25:3379-88; Dujon et al., *Gene* (1989) 82:115-8; Perler et al., *Nucleic Acids Res.* (1994) 22:1125-7; Jasin, *Trends Genet.* (1996) 12:224-8; Gimble et al., *J Mol Biol.* (1996) 263:163-80; Argast et al., *J Mol Biol.* (1998) 280:345-53; and the New

England Biolabs catalogue. In addition, the DNA-binding specificity of homing endonucleases and meganucleases can be engineered to bind non-natural target sites. *See*, for example, Chevalier et al., *Mol Cell* (2002) 10:895-905; Epinat et al., *Nucleic Acids Res.* (2003) 31:2952-62; Ashworth et al., *Nature* (2006) 441:656-59; Paques et al., *Current Gene Therapy* (2007) 7:49-66; and U.S. Pat. Pub. 2007/0117128.

III. Zinc-Finger Protein Transcription Factors

[0057] Zinc finger protein transcription factors for repressing PrP expression is disclosed in International Patent Publication WO 2021/067864, the entirety of which is incorporated by reference herein and specifically incorporated by reference in its entirety is the section related to Zinc Finger Protein Transcription Factors.

[0058] The ZFP domains described herein may be fused to a transcription factor. The ZFP domains described herein may be fused to an epigenetic silencing domain. In some embodiments, the transcription factor may be a transcription repressor domain, wherein the ZFP and repressor domains may be associated with each other by a direct peptidyl linkage or a peptide linker, or by dimerization (*e.g.*, through a leucine zipper, a STAT protein N terminal domain, or an FK506 binding protein). As used herein, a "fusion protein" refers to a polypeptide with covalently linked domains as well as a complex of polypeptides associated with each other through non-covalent bonds. The transcription repressor domain or epigenetic silencing domain can be associated with the ZFP domain at any suitable position, including the C- or N-terminus of the ZFP domain.

[0059] In some embodiments, two or more of the ZFRs are used concurrently in a cell, animal, subject, patient, where the ZFRs bind to different target regions in the *PRNP* gene, so as to achieve optimal repression of *PRNP* expression.

A. Transcription Repressor Domains

[0060] Zinc finger protein transcription factors for repressing PrP expression is disclosed in International Patent Publication WO 2021/067864, the entirety of which is incorporated by reference herein and specifically incorporated by reference in its entirety is the section related to Zinc Finger Repressor Domains.

[0061] The ZFRs comprise an engineered ZFP domain as described herein and one or more transcription repressor domain or epigenetic silencing domain that dampen the transcription activity of the *PRNP* gene. One or more engineered ZFP domains and one or more transcription repressor domains may be joined by a flexible linker. Non-limiting examples of transcription repressor domains and epigenetic silencing domains are the KOX1 KRAB

domain, KAP-1, MAD, FKHR, EGR-1, ERD, SID, TGF-beta-inducible early gene (TIEG), v-ERB-A, MBD2, MBD3, TRa, histone methyltransferase, histone deacetylase (HDAC), nuclear hormone receptor (*e.g.*, estrogen receptor or thyroid hormone receptor), members of the DNMT family (*e.g.*, DNMT1, DNMT3A, DNMT3B, DNMT3L, or DNMT3L with a Histone 3 tail), Rb, and MeCP2. *See, e.g.*, Bird et al. (1999) *Cell* 99:451-454; Tyler *et al.* (1999) *Cell* 99:443-446; Knoepfler *et al.* (1999) *Cell* 99:447-450; Robertson *et al.* (2000) *Nature Genet.* 25:338-342; Neumann *et al.* (2024) *Science*. 384: ado7082. Additional exemplary repression domains include, but are not limited to, ROM2 and AtHD2A. See, for example, Chem *et al.* (1996) *Plant Cell* 8:305-321; and Wu *et al.* (2000) *Plant J.* 22:19-27.

[0062] In some embodiments, the transcription repressor domain or epigenetic silencing domain comprises a sequence from the Kruppel-associated box (KRAB) domain of the human zinc finger protein 10/KOX1 (ZNF10/KOX1) (*e.g.*, GenBank No. NM_015394.4). An exemplary KRAB domain sequence is:

DAKSLTAWSR TLVTFKDVFV DFTREEWKLL DTAQQIVYRN
VMLENYKNLV SLGYQLTKPD VILRLEKGEE PWLVEREIHQ ETHPDSETAF
EIKSSV
(SEQ ID NO: 261).

Variants of this KRAB sequence may also be used so long as they have the same or similar transcription repressor function. For example, any one of the KRAB domains as described in Alerasool, N., Segal, D., Lee, H. et al. An efficient KRAB domain for CRISPRi applications in human cells. Nat Methods 17, 1093–1096 (2020). https://doi.org/10.1038/s41592-020-0966-x.

[0063] In some embodiments, an engineered ZFR described herein binds to a target site as shown in a single row of Tables 1 and 3, preferably with no or little detectable off-target binding or activity. Off-target binding may be determined, for example, by measuring the activity of ZFRs at off-target genes.

[0064] In some embodiments, an engineered ZFR described herein comprises a DNA-binding recognition helix sequence shown in Tables 1 and 3. In some embodiments, an engineered ZFR described herein comprises two adjacent DNA-binding recognition helix sequences shown in a single row in Tables 1 and 3. In some embodiments, an engineered ZFR described herein comprises the DNA-binding recognition helix sequences shown in a single row in Tables 1 and 3**.** In some embodiments, an engineered ZFR described herein comprises the recognition helix and backbone portions of a sequence shown in a single row in Tables 2

and 4. In some embodiments, an engineered ZFR described herein comprises an amino acid sequence as shown in a single row in Tables 2 and 4.

[0065] In some embodiments, an engineered ZFR described herein comprises the recognition helix and backbone portions of a sequence shown in a single row in Tables 2 and 4 as the sequence would appear following post-translational modification. In some embodiments, an engineered ZFR described herein comprises an amino acid sequence as shown in a single row in Tables 2 and 4, as the sequence would appear following post-translational modification. For example, post-translational modification may remove the initiator methionine residue from a sequence as shown in a single row in Tables 2 and 4.

B. Peptide Linkers

[0066] Zinc finger protein transcription factors for repressing PrP expression is disclosed in International Patent Publication WO 2021/067864, the entirety of which is incorporated by reference herein and specifically incorporated by reference in its entirety is the section related to Peptide linkers.

[0067] The ZFP domain and the transcription repressor domain of the ZFRs and/or the zinc fingers within the ZFP domains may be linked through a peptide linker, *e.g.*, a noncleavable peptide linker of about 5 to 50 amino acids (*e.g.*, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acids). Preferred linkers are typically flexible amino acid subsequences that are synthesized as a recombinant fusion protein. In some embodiments, zinc fingers are linked such that there is no gap between the linked module target subsites in the target nucleic acid molecule. In other embodiments, zinc fingers are linked by linkers designed to allow the linked modules to bind to target sites with 1, 2 or 3 base pair gaps between the linked module target subsites in the target nucleic acid molecule. *See*, *e.g.*, U.S. Patent No. 8,772,453.

[0068] In some embodiments, the peptide linker is 3 to 20 amino acid residues in length and is rich in G and/or S. Non-limiting examples of such linkers are G_4S-type linkers, *i.e.*, linkers containing one or more (*e.g.*, 2, 3, or 4) GGGGS (SEQ ID NO: 15) motifs, or variations of the motif (such as ones that have 1, 2, or 3 amino acid insertions, deletions, and substitutions from the motif).

[0069] Linker design methods and illustrative linkers that may be used to link the ZFP domain and the transcription repressor domain of the ZFRs and/or the zinc fingers within the ZFP domains are described in U.S. Patent NOS: 6,479,626 (herein incorporated by reference in its entirety); 7,851,216 (herein incorporated by reference in its entirety); 8,772,453 (herein incorporated by reference in its entirety); 9,394,531 (herein incorporated by reference

in its entirety); 9,567,609 (herein incorporated by reference in its entirety); and 10,724,020 (herein incorporated by reference in its entirety); and PCT Publication NOS: WO 1999/045132 (herein incorporated by reference in its entirety); WO 2001/053480 (herein incorporated by reference in its entirety); WO 2009/154686 (herein incorporated by reference in its entirety); WO 2011/139349 (herein incorporated by reference in its entirety); WO 2015/031619 (herein incorporated by reference in its entirety); and WO 2017/136049 (herein incorporated by reference in its entirety). The proteins described herein may include any combination of suitable linkers.

[0070] Non-limiting examples of linkers are DGGGS (SEQ ID NO: 3), TGEKP (SEQ ID NO: 4), LRQKDGERP (SEQ ID NO: 5), GGRR (SEQ ID NO: 6), GGRRGGGS (SEQ ID NO: 7), LRQRDGERP (SEQ ID NO: 8), LRQKDGGGSERP (SEQ ID NO: 9), LRQKD$(G_3S)_2$ ERP (SEQ ID NO: 10), TGSQKP (SEQ ID NO: 11), LRQKDAARGS (SEQ ID NO: 13), and LRQKDAARGSGG (SEQ ID NO: 14). Additional illustrative linkers for linking zinc fingers and/or for linking domains are listed in Table 5. The finger-finger linkers listed in Table 5 include portions of backbone sequence, *e.g.*, FQ or FA.

[0071] Table 5 shows illustrative alternate peptide linkers that may be used to link zinc finger amino acid sequences and/or ZFP and functional domain sequences as shown in Tables 1 and Table 3.

Table 5

Linker Position	Linker Category	Exemplary Linker		
		Linker Code	Peptide sequence	SEQ ID NO:
Finger-Finger	No base skipping	0a	TGEKPFQ	93
		0b	TGGQRPFQ	94
		0c	TGSQKPFQ	95
		0d	TGSQRPFQ	96
		0f	TGEKPFA	97
	1 base skipping	1a	TGGGGSQRPFQ	98
		1b	TGGGGSQKPFQ	99
		1c	THPRAPIPKPFQ	100
		1d	TPNRRPAPKPFQ	101
		1e	TVPRPTPPKPFQ	102
		1f	TYPRPIAAKPFQ	103
	2 base skipping	2a	TGGGGSGGSQRPFQ	104
		2b	TGGGGSGGSQKPFQ	105
		2d	TLAPRPYRPPKPFQ	106
		2e	TPGGKSSRTDRNKPFQ	107
		2f	TPNPHRRTDPSHKPFQ	108

ZFP-functional domain (interdomain)	ZFR	C0	LRGSGG	109
		C1	LRQKDAARGS	13
		C1k	LRQKDAARGSGG	14

IV. Expression of the ZFRs

[0072] Zinc finger protein transcription factors for repressing PrP expression is disclosed in International Patent Publication WO 2021/067864, the entirety of which is incorporated by reference herein and specifically incorporated by reference in its entirety is the section related to Expression of the ZFRs.

[0073] A ZFR may be introduced to a patient through a nucleic acid molecule encoding it. The nucleic acid molecule may be an RNA or cDNA molecule. The nucleic acid molecule may be introduced into the brain of the patient through injection of a composition comprising a lipid:nucleic acid complex (*e.g.*, a liposome). Alternatively, the ZFR may be introduced to the patient through a nucleic acid expression vector comprising a sequence encoding the ZFR. The expression vectors may include expression control sequences such as promoters, enhancers, transcription signal sequences, and transcription termination sequences that allow expression of the coding sequence for the ZFRs in the cells of the nervous system. In some embodiments, the expression vector remains present in the cell as a stable episome. In other embodiments, the expression vector is integrated into the genome of the cell.

[0074] In some embodiments, the promoter on the vector for directing the ZFR expression in the brain is a constitutive active promoter or an inducible promoter. Suitable promoters include, without limitation, a retroviral RSV LTR promoter (optionally with an RSV enhancer), a CMV promoter (optionally with a CMV enhancer), a CMV immediate early promoter, an SV40 promoter, a dihydrofolate reductase (DHFR) promoter, a β-actin promoter, a phosphoglycerate kinase (PGK) promoter, an EFlα promoter, a MoMLV LTR, a CK6 promoter, a TK promoter, a tetracycline responsive promoter (TRE), an HBV promoter, chimeric liver-specific promoters (LSPs), an E2F promoter, the telomerase (hTERT) promoter, a CMV enhancer/chicken β-actin/rabbit β-globin promoter (CAG promoter; Niwa et al., *Gene* (1991) 108(2):193-9), and an RU-486-responsive promoter. Neuron- or glial-specific promoters such as a Synapsin I promoter, a CAMKII promoter, a MeCP2 promoter, a PrP promoter, a GFAP promoter, or an engineered or natural promoter that restricts expression to neuron and glial cells may also be used.

[0075] Any method of introducing the nucleotide sequence into a cell may be employed, including but not limited to, electroporation, calcium phosphate precipitation,

microinjection, cationic or anionic liposomes, liposomes in combination with a nuclear localization signal, naturally occurring liposomes (*e.g.*, exosomes), or viral transduction.

[0076] For *in vivo* delivery of a *PRNP*-targeting ZFR fusion protein, viral transduction is used using an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding the fusion protein. For *in vivo* delivery of an expression vector, viral transduction may be used. A variety of viral vectors known in the art may be adapted by one of skill in the art for use in the present disclosure, for example, vaccinia vectors, adenoviral vectors, lentiviral vectors, poxyviral vectors, adeno-associated viral (AAV) vectors, retroviral vectors, and hybrid viral vectors. In some embodiments, the viral vector used herein is a recombinant AAV (rAAV) vector. AAV vectors are especially suitable for CNS gene delivery because they infect both dividing and non-dividing cells, exist as stable episomal structures for long-term expression, and have very low immunogenicity (Hadaczek et al., *Mol Ther*. (2010) 18:1458-61; Zaiss, et al., *Gene Ther*. (2008) 15:808-16). Any suitable AAV serotype may be used. For example, the AAV may be AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV8.2, AAV9, or AAVrh10, or of a pseudotype such as AAV2/8, AAV2/5, AAV2/6 or AAV2/9 (*i.e.*, AAV derived from multiple serotypes; for example, the rAAV comprises AAV2 inverted terminal repeats (ITR) in its genome and an AAV8, 5, 6, or 9 capsid). In some embodiments, the expression vector is an AAV viral vector and is introduced to the target human cell by a recombinant AAV virion whose genome comprises the construct, including having the AAV Inverted Terminal Repeat (ITR) sequences on both ends to allow the production of the AAV virion in a production system such as an insect cell/baculovirus production system or a mammalian cell production system). The AAV may be engineered such that its capsid proteins have reduced immunogenicity, enhanced transduction ability, and/or an augmented ability to cross a biological barriers (such as the blood brain barrier) in humans, nonhuman primates, or rodents. In some embodiments, AAV9 is used. Viral vectors described herein may be produced using methods known in the art. Any suitable permissive or packaging cells may be employed to produce the viral particles. For example, mammalian or insect cells may be used as the packaging cell line.

[0077] In some embodiments, a prion-targeting transcriptional repressor (*e.g.*, ZFR) is delivered by STAC-BBB. In some embodiments, any one of the ZFRs described herein are delivered by a BBB penetrant AAV capsid. In some embodiments the BBB penetrant AAV capsid is STAC-BBB.

[0078] In some embodiments, targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) is introduced into the AAV capsid

proteins VP1, VP2 or VP3, or in two of the capsid proteins in any combination, or in all three. In some embodiments, the targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) is introduced into VP1. In some embodiments, the targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) is introduced into VP2. In some embodiments, the targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) is introduced into VP3. In some embodiments, the targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) is introduced into VP1 and VP2. In some embodiments, the targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) is introduced into VP1 and VP3. In some embodiments, the targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) is introduced into VP2 and VP3. In some embodiments, the targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) is introduced into VP1, VP2, and VP3. In some embodiments, the targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) is introduced at a single site in a capsid protein such as 1496 or 1497.

[0079] In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) introduced into the AAV capsid proteins VP1, VP2 or VP3, or in two of the AAV capsid proteins VP1, VP2 or VP3 in any combination, or in all three. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) introduced into VP1. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) introduced into VP2. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) introduced into VP3. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) introduced into VP1 and VP2. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) introduced into VP1 and VP3. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) are introduced into VP2 and VP3. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ ID NOs: 333, 417, 442, or 673) introduced into VP1, VP2, and VP3. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 263-1495, preferably one of SEQ

ID NOs: 333, 417, 442, or 673) is introduced at a single site in a capsid protein such as 1496 or 1497.

[0080] In some embodiments, the AAV capsid protein comprises one of SEQ ID NO: 333, 417, 442 or 673 introduced into the AAV capsid proteins VP1, VP2 or VP3. In some embodiments, the AAV capsid protein comprises one of SEQ ID NO: 333, 417, 442 or 673 introduced into two of the AAV capsid proteins VP1, VP2 or VP3 in any combination. In some embodiments, the AAV capsid protein comprises one of SEQ ID NO: 333, 417, 442 or 673 introduced into the AAV capsid proteins VP1, VP2 and VP3. In some embodiments, the AAV capsid protein comprises one of SEQ ID NO: 333, 417, 442 or 673 introduced into VP1 and not VP2 or VP3. In some embodiments, the AAV capsid protein comprises one of SEQ ID NO: 333, 417, 442 or 673 introduced into VP2 and not VP1 or VP3. In some embodiments, the AAV capsid protein comprises one of SEQ ID NOs: 333, 417, 442, or 673introduced into VP3 and not VP2 or VP1. In some embodiments, the AAV capsid protein comprises one of SEQ ID NOs: 333, 417, 442, or 673introduced into VP1 and VP2 and not VP3. In some embodiments, the AAV capsid protein comprises one of SEQ ID NOs: 333, 417, 442, or 673introduced into VP1 and VP3 and not VP2. In some embodiments, the AAV capsid protein comprises one of SEQ ID NOs: 333, 417, 442, or 673introduced into VP2 and VP3 and not VP1. In some embodiments, the AAV capsid protein comprises one of SEQ ID NOs: 333, 417, 442, or 673introduced into VP1, VP2, and VP3. In some embodiments, the AAV capsid protein comprises one of SEQ ID NOs: 333, 417, 442, or 673is introduced at a single site in a capsid protein such as 1496 or 1497.

[0081] In some embodiments, the AAV capsid protein comprises SEQ ID NOS: 1498, 1499 or 1500. In some embodiments, the AAV capsid protein comprises SEQ ID NOS: 1498, 1499 and 1500. In some embodiments, the AAV capsid protein comprises SEQ ID NOS: 1498 or 1499 and not 1500. In some embodiments, the AAV capsid protein comprises SEQ ID NOS: 1498 or 1500 and not 1499. In some embodiments, the AAV capsid protein comprises SEQ ID NOS: 1499 or 1500 and not 1498. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 1498 and not 1499 or 1500. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 1499 and not 1498 or 1500. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 1500 and not 1499 or 1498.

[0082] In some embodiments, the composition comprises an AAV capsid protein, the AAV capsid protein comprising SEQ ID NOS: 1498, 1499 or 1500 and an expression construct comprising a coding sequence for a fusion protein, wherein the fusion protein comprises a zinc finger protein (ZFP) domain and a transcription repressor domain, wherein

the ZFP domain binds to a target region of a human *PRNP* gene. In some embodiments, the composition comprises an AAV capsid protein the AVV capsid protein comprising SEQ ID NOS: 1498, 1499 and 1500 and an expression construct comprising a coding sequence for a fusion protein, wherein the fusion protein comprises a zinc finger protein (ZFP) domain and a transcription repressor domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene. In some embodiments, the composition comprises an AAV capsid protein, the AAV capsid protein comprising SEQ ID NOS: 1498 or 1499 and an expression construct comprising a coding sequence for a fusion protein, wherein the fusion protein comprises a zinc finger protein (ZFP) domain and a transcription repressor domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene. In some embodiments, the composition comprises an AAV capsid protein, the AAV capsid protein comprises SEQ ID NOS: 1498 or 1500 and an expression construct comprising a coding sequence for a fusion protein, wherein the fusion protein comprises a zinc finger protein (ZFP) domain and a transcription repressor domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene. In some embodiments, the composition comprises an AAV capsid protein, the AAV protein comprises SEQ ID NOS: 1499 or 1500 and an expression construct comprising a coding sequence for a fusion protein, wherein the fusion protein comprises a zinc finger protein (ZFP) domain and a transcription repressor domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene. In some embodiments, the composition comprises an AAV capsid protein, the AAV capsid protein comprises SEQ ID NO: 1498 and not 1499 or 1500 and an expression construct comprising a coding sequence for a fusion protein, wherein the fusion protein comprises a zinc finger protein (ZFP) domain and a transcription repressor domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene. In some embodiments, the composition comprises an AAV capsid protein, the AAV capsid protein comprises SEQ ID NO: 1499 and not 1498 or 1500 and an expression construct comprising a coding sequence for a fusion protein, wherein the fusion protein comprises a zinc finger protein (ZFP) domain and a transcription repressor domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene. In some embodiments, the composition comprises an AAV capsid protein, the AAV capsid protein comprises SEQ ID NO: 1500 and not 1499 or 1498 and an expression construct comprising a coding sequence for a fusion protein, wherein the fusion protein comprises a zinc finger protein (ZFP) domain and a transcription repressor domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene.

[0083] In some embodiments, an expression construct encoding the ZFRs described herein is packaged into an AAV capsid comprising a AAV capsid sequence shown below in

SEQ ID. NO: 1498 encapsulates an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 (See FIG. 1B). In some embodiments, an expression construct encoding the ZFRs described herein is packaged into an AAV capsid comprising a AAV capsid sequence shown below in SEQ ID. NO: 1498 encapsulating an expression construct encoding *PRNP* ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 (See FIG. 1B). In some embodiments, an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 is packaged into an AAV capsid comprising an AAV capsid protein selected from the group comprising, consisting, or consisting essentially of SEQ ID. NO: 1498, SEQ ID. NO: 1499, SEQ ID. NO: 1500 and combinations thereof encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3. In some embodiments, an expression construct encoding a *PRNP* ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 is packaged into an AAV capsid comprising an AAV capsid protein selected from the group comprising, consisting, or consisting essentially of SEQ ID. NO: 1498, SEQ ID. NO: 1499, SEQ ID. NO: 1500 and combinations thereof encapsulating an expression construct encoding a fusion protein comprising a *PRNP* ZFR from Tables 2 and 4. In some embodiments, an expression construct encoding the ZFRs described herein is packaged into an AAV capsid comprising all of SEQ ID. NO: 1498, SEQ ID. NO: 1499, SEQ ID. NO: 1500. In some embodiments, an expression construct encoding the ZFRs described herein is packaged into an AAV capsid comprising one of SEQ ID. NO: 1498, SEQ ID. NO: 1499, SEQ ID. NO: 1500. In some embodiments, an expression construct encoding the ZFRs described herein is packaged into an AAV capsid comprising a plurality of SEQ ID. NO: 1498, SEQ ID. NO: 1499, SEQ ID. NO: 1500.

[0084] In some embodiments, a variant AAV capsid protein sequence may be used. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1498. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1499. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1500. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NOS: 1498, 1499 and 1500. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1498 and encapsulates an expression

construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1498 and encapsulates an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising a ZFR as shown in a single row in Tables 2 and 4. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1499 and encapsulates an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1499 and encapsulates an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising a ZFR as shown in a single row in Tables 2 and 4. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1500 and encapsulates an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1500 and encapsulates an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising a ZFR as shown in a single row in Tables 2 and 4. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NOS: 1498, 1499 and 1500 and encapsulates an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NOS: 1498, 1499 and 1500 and encapsulates an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising a ZFR as shown in a single row in Tables 2 and 4.

[0085] In some embodiments, a vector encoding any one of the ZFP or ZFR sequences as shown in Tables 1-4 is packaged into SEQ ID NO. 1497. In some embodiments, a vector encoding any one of the ZFP or ZFR sequences as shown in Tables 1-4 is packaged into SEQ ID NO. 1496. In some embodiments, a vector encoding any one of the ZFP or ZFR sequences as shown in a single row of Tables 1-4 is packaged into an AAV.

[0086] Here, the novelty is the combination of a *PRNP* targeting ZFP delivered via a novel AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of

an amino acid sequence set forth in any one of SEQ ID NO: 263-1495. An AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 is not the same as an AAV. As discussed above, an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 (and in particular SEQ ID 1498) is a specifically modified AAV. In some embodiments, the novel AAV capsid protein has an insertion of a peptide sequence into variable region 8 of the AAV capsid. In some embodiments, the novel AAV capsid protein has an insertion of a peptide sequence into variable region 8 of the AAV capsid and is capable of crossing the blood brain barrier in non-human primates and humans. In some embodiments, the specific sequence of the novel AAV capsid protein has at least 80%, 85%, 90%, 95%, or 99% identity to a sequence shown in SEQ ID NOS: 1498, 1499, or 1500.

[0087] An AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in one of SEQ ID NOs: 333, 417, 442, or 673 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 can facilitate movement of the construct across the blood brain barrier. An AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in one of SEQ ID NOs: 333, 417, 442, or 673 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising a ZFR as shown in a single row in Tables 2 and 4 can facilitate movement of the construct across the blood brain barrier. The AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in one of SEQ ID NOs: 333, 417, 442, or 673 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 generally increases or enhances movement of the construct across the blood brain barrier compared to SEQ ID Nos 1496 or 1497. The AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in one of SEQ ID NOs: 333, 417, 442, or 673 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising a ZFR as shown in a single row in Tables 2 and 4 generally increases or enhances movement of the construct across the blood brain barrier compared to SEQ ID Nos 1496 or 1497. The efficiency of movement across the blood brain barrier can be increased by at least or about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or

more, *e.g.* an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 can be at least or about 1.2×, 1.5×, 2×, 3×, 4×, 5×, 6×, 7×, 8×, 9×, 10×, 11×, 12×, 13×, 14×, 15×, 16×, 17×, 18×, 19×, 20×, 30×, 40×, 50×, 60×, 70×, 80×, 90×, 100× or more compared to a non-modified AAV capsid polypeptide (*e.g.* SEQ ID NO: 1496 or 1497). The efficiency of movement across the blood brain barrier can be increased by at least or about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more, *e.g.* an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 can be at least or about 1.2×, 1.5×, 2×, 3×, 4×, 5×, 6×, 7×, 8×, 9×, 10×, 11×, 12×, 13×, 14×, 15×, 16×, 17×, 18×, 19×, 20×, 30×, 40×, 50×, 60×, 70×, 80×, 90×, 100× or more compared to a non-modified AAV capsid polypeptide (*e.g.* SEQ ID NO: 1496 or 1497). In particular examples, the increased movement of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 across the blood brain barrier is observed in non-human primates and humans *in vivo*. In particular examples, the increased movement of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 across the blood brain barrier is observed in non-human primates and humans *in vivo*. An AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 is therefore particularly useful in delivering ZFPs to the brain, such as for therapy of a brain-associated disease or condition. An AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 is therefore particularly useful in delivering ZFPs to the brain, such as for therapy of a brain-associated disease or condition. In some embodiments, the ZFP from the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 is administered to a subject and is

not expressed outside the brain. In some embodiments, the ZFP from the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising a ZFR as shown in a single row in Tables 2 and 4 is administered to a subject and is not expressed outside the brain. In some embodiments, the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 comprises, consists, or consists essentially of a hSYN1 promoter that is administered to a subject and is not expressed outside the brain. In some embodiments, the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising a ZFR as shown in a single row in Tables 2 and 4 comprises, consists, or consists essentially of a hSYN1 promoter that is administered to a subject and is not expressed outside the brain. In some embodiments, the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 comprising, consisting, or consisting essentially of a hSYN1 promoter is administered to a subject and is not expressed outside the brain and is administered to the subject by an intravenous, intrathecal, intracerebral, intracerebroventricular, intra-cisternal magna, intrahippocampal, intrathalamic, or intraparenchymal route. In some embodiments, the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising a ZFR as shown in a single row in Tables 2 and 4 comprising, consisting, or consisting essentially of a hSYN1 promoter is administered to a subject and is not expressed outside the brain and is administered to the subject by an intravenous, intrathecal, intracerebral, intracerebroventricular, intra-cisternal magna, intrahippocampal, intrathalamic, or intraparenchymal route. In some embodiments, the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct an expression construct encoding a

PRNP targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 comprising, consisting, or consisting essentially of a hSYN1 promoter is administered to a subject and is not expressed outside the brain and is administered to the subject by an intravenous, intrathecal, intracerebral, intracerebroventricular, intra-cisternal magna, intrahippocampal, intrathalamic, or intraparenchymal route at a dose between 1E12 vg/kg to 5E14 vg/kg or therapeutically effective amount. In some embodiments, the AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising a ZFR as shown in a single row in Tables 2 and 4 comprising, consisting, or consisting essentially of a hSYN1 promoter is administered to a subject and is not expressed outside the brain and is administered to the subject by an intravenous, intrathecal, intracerebral, intracerebroventricular, intra-cisternal magna, intrahippocampal, intrathalamic, or intraparenchymal route at a dose between 1E12 vg/kg to 5E14 vg/kg or therapeutically effective amount. In some embodiments, the subject is a mammal preferably a human.

[0088] An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NO: 1498 encapsulating a heterologous coding sequence the heterologous coding sequence coding a fusion protein comprising, consisting, or consisting essentially of a zinc finger protein (ZFP) domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the ZFP domain comprises DNA-binding recognition helix sequences as shown in a single row in Tables 1 and 3. An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NO: 1498 encapsulating a heterologous coding sequence the heterologous coding sequence coding a fusion protein comprising, consisting, or consisting essentially of an amino acid sequence as shown in a single row in Tables 2 and 4.

[0089] An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NO: 1499 and a heterologous coding sequence the heterologous coding sequence coding a fusion protein comprising, consisting, or consisting essentially of a zinc finger protein (ZFP) domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the ZFP domain comprises DNA-binding recognition helix sequences as shown in a single row in Tables 1 and 3. An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NO: 1499 and a heterologous coding sequence the heterologous coding sequence coding a fusion protein comprising, consisting, or consisting essentially of an amino acid sequence as shown in a single row in Tables 2 and 4.

[0090] An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NO: 1500 and a heterologous coding sequence the heterologous coding sequence coding a fusion protein comprising, consisting, or consisting essentially of a zinc finger protein (ZFP) domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the ZFP domain comprises DNA-binding recognition helix sequences as shown in a single row in Tables 1 and 3. An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NO: 1500 and a heterologous coding sequence the heterologous coding sequence coding a fusion protein comprising, consisting, or consisting essentially of an amino acid sequence as shown in a single row in Tables 2 and 4.

[0091] An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NOS: 1498, 1499, 1500 and combinations thereof and a heterologous coding sequence the heterologous coding sequence coding a fusion protein comprising, consisting, or consisting essentially of a zinc finger protein (ZFP) domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the ZFP domain comprises DNA-binding recognition helix sequences as shown in a single row in Tables 1 and 3. An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NOS: 1498, 1499, 1500 and combinations thereof and a heterologous coding sequence the heterologous coding sequence coding a fusion protein comprising, consisting, or consisting essentially of an amino acid sequence as shown in a single row in Tables 2 and 4.

[0092] An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NOS: 1498, 1499, 1500 and combinations thereof and a *PRNP* ZFR fused to the KRAB repression domain derived from the human *ZNF10* gene. An embodiment comprises a composition comprising a capsid polypeptide comprising SEQ ID NOS: 1498, 1499, 1500 and combinations thereof and a *PRNP* ZFR fused to the KRAB repression domain derived from the human *ZNF10* gene and cloned downstream of the hSYN1 promoter to restrict the expression of the ZFR to neurons. An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NOS: 1498, 1499, 1500 and combinations thereof and a *PRNP* ZFR fused to the KRAB repression domain derived from the human *ZNF10* gene and cloned downstream of the hSYN1 promoter and flanked by AAV2 inverted terminal repeat sequences.

[0093] An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NOS: 1498, 1499, 1500 and combinations thereof encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 fused to the

KRAB repression domain derived from the human *ZNF10* gene. An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NOS: 1498, 1499, 1500 and combinations thereof encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 fused to the KRAB repression domain derived from the human *ZNF10* gene. An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NOS: 1498, 1499, 1500 and combinations thereof encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 fused to the KRAB repression domain derived from the human *ZNF10* gene and cloned downstream of the hSYN1 promoter to restrict the expression of the ZFP to neurons. An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NOS: 1498, 1499, 1500 and combinations thereof encapsulating an expression construct encoding a fusion protein comprising a ZFRZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 fused to the KRAB repression domain derived from the human *ZNF10* gene and cloned downstream of the hSYN1 promoter to restrict the expression of the ZFP to neurons. An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NOS: 1498, 1499, 1500 and combinations thereof encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 fused to the KRAB repression domain derived from the human *ZNF10* gene and cloned downstream of the hSYN1 promoter and flanked by AAV2 inverted terminal repeat sequences. An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NOS: 1498, 1499, 1500 and combinations thereof encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 fused to the KRAB repression domain derived from the human *ZNF10* gene and cloned downstream of the hSYN1 promoter and flanked by AAV2 inverted terminal repeat sequences.

[0094] Disclosed is a method for producing an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495, comprising transfecting a host cell with three plasmids – (i) an AAV Helper plasmid containing the AAV2 Rep and STAC-BBB Cap genes, (ii) an Adenovirus Helper plasmid containing the adenovirus helper genes, and (iii) a transgene plasmid containing the sequence to be packaged flanked by AAV2 inverted terminal repeats, under conditions

suitable to facilitate assembly of an AAV vector comprising a capsid comprising, consisting, or consisting essentially of SEQ ID NOS: 1498, 1499, and/or 1500, wherein the capsid encapsulates an expression construct encoding a fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene. In some embodiments, the ZFP domain comprises, consists, or consists essentially of a DNA-binding recognition helix sequence as shown in Tables 1 and 3. In some embodiments, the ZFP domain comprises, consists, or consists essentially of DNA-binding recognition helix sequences linked as shown in Tables 2 and 4. In some embodiments, the ZFP domain comprises, consists, or consists essentially of DNA-binding recognition helix sequences as shown in a single row in Tables 1 and 3. In some embodiments, the ZFP domain binds to a Target Sequence as shown in a single row in Tables 1 and 3. In some embodiments, the fusion protein comprises, consists, or consists essentially of a sequence as shown in a single row in Tables 2 and 4. In some embodiments, the fusion protein comprises a DNA-binding recognition helix sequence having at least 80%, 85%, 90% or 99% identity to a sequence as shown in a single row of Tables 1 and 3. In some embodiments, the fusion protein comprises a sequence having at least 80%, 85%, 90% or 99% identity to a sequence as shown in a single row in Tables 2 and 4. In some embodiments, the fusion protein comprises, consists, or consists essentially of a sequence as shown in a single row in Tables 2 and 4. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 1498.

[0095] Disclosed are methods of administering an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein (*e.g.,* the AAV capsid protein comprises SEQ ID NO: 1498 and the *PRNP* targeted ZFP fusion protein, the fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3) to a subject. Disclosed are methods of administering an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 encapsulating an expression construct encoding a *PRNP* targeted ZFP fusion protein (*e.g.,* the AAV capsid protein comprises SEQ ID NO: 1498 and the *PRNP* targeted ZFP fusion protein comprises a sequence as shown in a single row in Tables 2 and 4) to a subject. In some embodiments, the subject is a human. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, ZFP expression is present in the right brain hemisphere. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, ZFP expression is present in the left brain hemisphere. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after

administration, ZFP expression is present in the right and left brain hemisphere. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, ZFP expression is present in the right or left brain hemisphere but not both. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, ZFP expression is not present outside the brain. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, ZFP expression is present in the right brain hemisphere across 10 brain levels. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, ZFP expression is present in the right brain hemisphere across 10 brain levels spanning 35 regions. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, ZFP expression is present in 7 coronal levels. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, ZFP expression is present across the rostrocaudal axis. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, ZFP expression is present inside and outside the brain. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, ZFP expression is present only inside the brain, not outside the brain. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, *PRNP* expression is reduced or stopped compared to prior to administration. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, *PRNP* expression is reduced or stopped compared to prior to administration at all levels and brain regions. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, *PRNP* expression is reduced or stopped at all levels and brain regions compared to prior to administration. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, ZFP expression is present in one or more brain cell selected from the group comprising, consisting, or consisting essentially of a motor neuron, a sensory neuron, a dopaminergic neuron, a cholinergic neuron, a glutamatergic neuron, a GABAergic neuron, or a serotonergic neuron, a glial cell, optionally an oligodendrocyte, an astrocyte, a pericyte, a Schwann cell, or a microglial cell, an ependymal cell, a neuroepithelial cell, or combinations thereof. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, *PRNP* expression is reduced or stopped compared to prior to administration in one or more brain cell selected from the group comprising, consisting, or consisting essentially of a motor neuron, a sensory neuron, a dopaminergic neuron, a cholinergic neuron, a glutamatergic neuron, a GABAergic neuron, or a serotonergic neuron, a glial cell, optionally an oligodendrocyte, an astrocyte, a pericyte, a Schwann cell, or a microglial cell, an ependymal cell, a neuroepithelial cell, or combinations thereof. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, ZFP expression is present in all brain regions. In some embodiments, 1-60 days,

preferably 28, 29, or 30 days after administration, *PRNP* expression is reduced or stopped compared to prior to administration in all brain regions. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, ZFP expression is present in one or more brain region selected from the group comprising, consisting, or consisting essentially of motor cortex, cortical regions, the entorhinal cortex, the hippocampus, the cerebellum, the globus pallidus, the thalamus, the midbrain, the caudate, the putamen, the substantia nigra, the pons, the medulla and combinations thereof. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, *PRNP* expression is reduced or stopped compared to prior to administration in one or more brain region selected from the group comprising, consisting, or consisting essentially of motor cortex, cortical regions, the entorhinal cortex, the hippocampus, the cerebellum, the globus pallidus, the thalamus, the midbrain, the caudate, the putamen, the substantia nigra, the pons, the medulla and combinations thereof.

[0096] In some embodiments, the AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 is targeted to different cell types and brain regions by altering the promoter, serotype, route of administration and combinations thereof. In some embodiments, expression of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 is restricted to brain cell types following administration. In some embodiments, expression of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 is not detected outside the brain following administration. In some embodiments, *PRNP* expression is reduced or stopped following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 compared to prior to administration only in brain cell types. In some embodiments, expression of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 is restricted to CNS cell types. In some embodiments, *PRNP* expression following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 is reduced or stopped compared to prior to administration only in

CNS cell types. In some embodiments, an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 has rapid pharmacokinetics, *i.e.,* 90-100% single cell potency. In some embodiments, the penetration discussed above is based on a one-time delivery of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3, ZFP expression is dose-dependent. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3, *PRNP* repression is dose-dependent. In some embodiments, the AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 dose is 1E14 vg/kg. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3, ZFP expression is present in individual neurons. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3, *PRNP* transcript levels in individual neurons is reduced or absent compared to prior to administration. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3, ZFP expression is present in in NeuN-positive cells. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3, *PRNP* transcript levels in individual neurons is reduced or absent in NeuN-positive cells compared to prior to

administration. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3, *PRNP* transcript levels in individual neurons is reduced or absent in NeuN-positive cells compared to prior to administration and ZFR signal is undetectable or minimally detected by ISH. In some embodiments, the AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 has a hSYN1 promoter and S100β-positive glial cells do not express ZFP but neurons have ZFP expression.

[0097] Disclosed is a method of treating a subject with a 1E14 vg/kg dose of, or between 1E12 vg/kg to 5E14 vg/kg, an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3. Following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3, the ZFP is expressed in cells selected from the group comprising, consisting, or consisting essentially of the pons region of the brainstem, cervical level of the spinal cord, precentral gyrus region of the motor cortex, temporal cortex, thalamus and combinations thereof. Following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3, the ZFP is expressed in cells selected from the group comprising, consisting, or consisting essentially of the pons region of the brainstem, cervical level of the spinal cord, precentral gyrus region of the motor cortex, temporal cortex, thalamus ChAT-positive motor neurons and combinations thereof. Following administration *PRNP* transcript levels in individual neurons is reduced compared to prior to administration. Following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3, *PRNP* transcript levels in individual neurons is reduced or absent compared to prior to administration. Following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3, *PRNP* transcript levels in individual neurons is reduced or absent

in NeuN-positive cells compared to prior to administration. Following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 *PRNP* transcript levels in individual neurons is reduced or absent in NeuN-positive cells compared to prior to administration and ZFR signal is undetectable or minimally detected by ISH. In some embodiments, the AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3 has a hSYN1 promoter and S100β-positive glial cells do not express ZFP but neurons have ZFP expression.

[0098] In some embodiments, the AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 is targeted to different cell types and brain regions by altering the promoter, serotype, route of administration and combinations thereof. In some embodiments, expression of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 is restricted to brain cell types following administration. In some embodiments, expression of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 is not detected outside the brain following administration. In some embodiments, *PRNP* expression is reduced or stopped following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 compared to prior to administration only in brain cell types. In some embodiments, expression of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 is restricted to CNS cell types. In some embodiments, *PRNP* expression following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 is reduced or stopped compared to prior to administration only in CNS cell types. In some embodiments, an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct

encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 has rapid pharmacokinetics, *i.e.,* 90-100% single cell potency. In some embodiments, the penetration discussed above is based on a one-time delivery of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4, ZFP expression is dose-dependent. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4, *PRNP* repression is dose-dependent. In some embodiments, the AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 dose is 1E14 vg/kg. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4, ZFP expression is present in individual neurons. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4, *PRNP* transcript levels in individual neurons is reduced or absent compared to prior to administration. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4, ZFP expression is present in in NeuN-positive cells. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4, *PRNP* transcript levels in individual neurons is reduced or absent in NeuN-positive cells compared to prior to administration. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after

administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4, *PRNP* transcript levels in individual neurons is reduced or absent in NeuN-positive cells compared to prior to administration and ZFR signal is undetectable or minimally detected by ISH. In some embodiments, the AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 has a hSYN1 promoter and S100β-positive glial cells do not express ZFP but neurons have ZFP expression.

[0099] Disclosed is a method of treating a subject with a 1E14 vg/kg dose of, or between 1E12 vg/kg to 5E14 vg/kg, an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4. Following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4, the ZFP is expressed in cells selected from the group comprising, consisting, or consisting essentially of the pons region of the brainstem, cervical level of the spinal cord, precentral gyrus region of the motor cortex, temporal cortex, thalamus and combinations thereof. Following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4, the ZFP is expressed in cells selected from the group comprising, consisting, or consisting essentially of the pons region of the brainstem, cervical level of the spinal cord, precentral gyrus region of the motor cortex, temporal cortex, thalamus ChAT-positive motor neurons and combinations thereof. Following administration *PRNP* transcript levels in individual neurons is reduced compared to prior to administration. Following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4, *PRNP* transcript levels in individual neurons is reduced or absent compared to prior to administration. Following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4, *PRNP* transcript levels in individual neurons is reduced or absent in NeuN-positive cells compared to prior to

administration. Following administration of an AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4 *PRNP* transcript levels in individual neurons is reduced or absent in NeuN-positive cells compared to prior to administration and ZFR signal is undetectable or minimally detected by ISH. In some embodiments, the AAV capsid protein comprising SEQ ID. NO: 1498 encapsulating an expression construct encoding a fusion protein comprising a ZFR, the ZFR comprising an amino acid sequence as shown in a single row in Tables 2 and 4has a hSYN1 promoter and S100β-positive glial cells do not express ZFP but neurons have ZFP expression.

[0100] V. CNS (Central Nervous System)-Targeting Molecules (Targeting Peptides)

[0101] Engineered blood brain barrier penetrant AAV capsids are disclosed in International Patent Publication WO 2024/238684, the entirety of which is incorporated by reference herein, and specifically incorporated by reference in its entirety is the section related to CNS (Central Nervous System)- Targeting Molecules (Targeting Peptides).

[0102] Described herein are CNS-targeting molecules, *i.e.,* targeting peptides. In some embodiments, the CNS-targeting molecules have enhanced tropism for a cell or tissue, such as the delivery of genetic material of interest to said cell or tissue, for example a CNS tissue or PNS tissue or a CNS cell or PNS cell.

[0103] In some embodiments, the CNS-targeting molecule comprises a sequence set forth in SEQ ID NO: 263-1495. In some embodiments, the targeting peptide comprises at least 5, 6, 7, 8, or 9 contiguous amino acids of a sequence set forth in SEQ ID NO: 263-1495. In some embodiments, the targeting peptide comprises the sequence set forth in SEQ ID NO: 333. In some embodiments, the targeting peptide comprises the sequence set forth in SEQ ID NO: 417. In some embodiments, the targeting peptide comprises the sequence set forth in SEQ ID NO: 442. In some embodiments, the targeting peptide comprises the sequence set forth in SEQ ID NO: 673. In some embodiments, the targeting peptide comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the sequence set forth in SEQ ID NO: 333. In some embodiments, the targeting peptide comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the sequence set forth in SEQ ID NO: 417. In some embodiments, the targeting peptide comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the sequence set forth in SEQ ID NO: 442. In some embodiments, the targeting peptide comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the sequence set forth in SEQ ID NO: 673.

[0104] In some embodiments, the CNS-targeting molecule comprises variants of the amino acid sequences set forth in SEQ ID NO: 263-1495. In embodiments, a variant refers to any one or more of a substitution, deletion, or addition to any of the amino acids of any of the amino acid sequences set forth in SEQ ID NO: 263-1495. In some embodiments, the variant comprises 1, 2, 3, or 4 substitutions to any of the amino acids of any of the amino acid sequences set forth in SEQ ID NO: 263-1495. In some embodiments, the variant comprises 1, 2, 3, or 4 deletions to any of the amino acids of any of the amino acid sequences set forth in SEQ ID NO: 263-1495. In some embodiments, the variant comprises 1, 2, 3, or 4 insertions to any of the amino acids of any of the amino acid sequences set forth in SEQ ID NO: 263-1495. In some embodiments, the variant comprises any combination of the substitutions, deletions, or insertions described above.

[0105] In embodiments, a variant refers to a variant in the nucleotide sequence that encodes any of the amino acid sequences set forth in SEQ ID NO: 263-1495. In embodiments, the variant in the nucleotide sequence results in encoding any one or more of a substitution, deletion, or addition to any of the amino acids of any of the amino acid sequences set forth in SEQ ID NO: 263-1495. In embodiments, the variant in the nucleotide sequence encodes 1, 2, 3, or 4 substitutions to any of the amino acids of any of the amino acid sequences set forth in SEQ ID NO: 263-1495. In embodiments, the variant in the nucleotide sequence encodes 1, 2, 3, or 4 deletions to any of the amino acids of any of the amino acid sequences set forth in SEQ ID NO: 263-1495. In embodiments, the variant in the nucleotide sequence encodes 1, 2, 3, or 4 insertions to any of the amino acids of any of the amino acid sequences set forth in SEQ ID NO: 263-1495. In embodiments, the variant in the nucleotide sequence encodes any combination of the substitutions, deletions, or insertions described above.

[0106] In some embodiments, the CNS-targeting molecule is fused or conjugated to a small molecule, an antibody, scFV, ASO (antisense oligonucleotide), siRNA, lipid, polymer or recombinant protein. In some embodiments, any of SEQ ID NO: 263-1495 are fused or conjugated to a small molecule, an antibody, scFV, ASO (antisense oligonucleotide), siRNA, lipid, polymer or recombinant protein. In some embodiments, one of SEQ ID NOS: 333, 417, 442, or 673 is fused or conjugated to a small molecule, an antibody, scFV, ASO (antisense oligonucleotide), siRNA, lipid, polymer or recombinant protein. In some embodiments, CNS-targeting molecules may be utilized to enable a small molecule, an antibody, scFV, ASO (antisense oligonucleotide), siRNA, lipid, polymer or recombinant protein to cross the blood brain barrier.

[0107] In some embodiments, the CNS-targeting molecules are part of an engineered AAV capsid protein. In some embodiments the engineered capsid protein comprises any of the serotypes of AAV1, AAV2, AAV3B, AAV5, AAV6, AAV8 and AAV9. In embodiments, the capsid protein comprises the serotype AAV2 or AAV9. In some embodiments, the AAV2 serotype comprises SEQ ID NO: 1496. In some embodiments, the AAV2 serotype comprises a variant of SEQ ID NO: 1496. In some embodiments, the AAV9 serotype comprises SEQ ID NO: 1497. In some embodiments, the AAV9 serotype comprises a variant of SEQ ID NO: 1497. In some embodiments, the AAV serotype comprises SEQ ID NOS: 1498, 1499, or 1500. In some embodiments, the AAV serotype comprises a variant of SEQ ID NOS: 1498, 1499, or 1500. In embodiments, a variant refers to any one or more of a substitution, deletion, or addition to any of the amino acids in either of the amino acid sequences set forth in SEQ ID NOs: 1496 or 1497. In embodiments, the variant comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 substitutions of any of the amino acids in either of the amino acid sequences of SEQ ID NOs: 1496 or 1497. In embodiments, the variant comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 deletions of any of the amino acids in either of the amino acid sequences set forth of SEQ ID NOs: 1496 or 1497. In embodiments, the variant comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 insertions of any of the amino acids in either of the amino acid sequences set forth SEQ ID NOs: 1496 or 1497.

[0108] In some embodiments, the variant comprises an amino acid sequence that comprises at least 80% sequence identity to the sequence set forth in SEQ ID NOs: 1496 or 1497 and at least 3, 4, 5, 6, 7, 8, 9 or all contiguous amino acids of any of SEQ ID NOS: 263-1495. In some embodiments, the variant comprises an amino acid sequence that comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NOs: 1496 or 1497 and at least 3, 4, 5, 6, 7, 8, 9 or all contiguous amino acids of any of SEQ ID NOS: 263-1495.

[0109] In some embodiments, the AAV2 serotype comprises a sequence that is at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical to SEQ ID NO: 1496. In some embodiments, the AAV9 serotype comprises a sequence that is at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical to SEQ ID NO: 1497. In some embodiments, the AAV9 serotype comprises a sequence that is at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical to

SEQ ID NO: 1498. In some embodiments, the AAV9 serotype comprises a sequence that is at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical to SEQ ID NO: 1499. In some embodiments, the AAV9 serotype comprises a sequence that is at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical to SEQ ID NO: 1500. In some embodiments, the AAV9 serotype comprises a sequence that is at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical to SEQ ID NOS: 1498, 1499, and 1500. In particular embodiments, the portion of the capsid polypeptide that is not the peptide modification comprises at least or about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, or 96% sequence identity to the AAV9 serotype set forth in SEQ ID NO: 1497.

[0110] In embodiments, a variant refers to a variant in the nucleotide sequence that encodes the amino acid sequences set forth in SEQ ID NOs: 1498, 1499, or 1500. In some embodiments, the variant in the nucleotide sequences results in encoding any one or more of a substitution, deletion, or addition to any of the amino acids of either of the amino acid sequences set forth in SEQ ID NOs: 1498, 1499, or 1500. In embodiments, the variant in the nucleotide sequence encodes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 substitutions of any of the amino acids in either of the amino acid sequences of SEQ ID NOs: 1496, or 1497. In embodiments, the variant in the nucleotide sequence encodes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 deletions of any of the amino acids in either of the amino acid sequences of SEQ ID NOS: 1496 or 1497. In some embodiments, the variant in the nucleotide sequence encodes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 insertions of any of the amino acids in either of the amino acid sequences of SEQ ID NOS: 1496, or 1497.

[0111] In some embodiments, the variant in the nucleotide sequence encodes an amino acid sequence that comprises at least 80% sequence identity to the sequence set forth in SEQ ID NOS: 1496 or 1497 and at least 3, 4, 5, 6, 7, 8, 9 or all contiguous amino acids of any of SEQ ID NOS: 263-1495. In some embodiments, the variant in the nucleotide sequence encodes an amino acid sequence that comprises at least 80%, 85%, 90%, 95% or 99% sequence identity to the sequence set forth in SEQ ID NOS: 1496 or 1497 and at least 3, 4, 5, 6, 7, 8, 9 or all contiguous amino acids of any of SEQ ID NOS: 263-1495.

[0112] In some embodiments, a nucleotide sequence encodes an amino acid sequence that is at least 80% identical, at least 85% identical, at least 90% identical, or at least

95% identical to SEQ ID NO: 1496 and has at least 3, 4, 5, 6, 7, 8, 9 or all contiguous amino acids of any of SEQ ID NOS: 263-1495. In some embodiments, a nucleotide sequence encodes an amino acid sequence that is at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical to SEQ ID NO: 1497 and has at least 3, 4, 5, 6, 7, 8, 9 or all contiguous amino acids of any of SEQ ID NOS: 263-1495. In some embodiments, a nucleotide sequence encodes an amino acid sequence that is at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical to SEQ ID NO: 1498. In some embodiments, a nucleotide sequence encodes an amino acid sequence that is at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical to SEQ ID NO: 1499. In some embodiments, a nucleotide sequence encodes an amino acid sequence that is at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical to SEQ ID NO: 1500.

[0113] In some embodiments, any of the CNS-targeting molecules set forth in any of SEQ ID NO: 263-1495 is inserted into an AAV. In some embodiments, any of the CNS-targeting molecules set forth in any of SEQ ID NO: 263-1495 is inserted into AAV2. In some embodiments, AAV2 comprises the sequence set forth in SEQ ID NO: 1496. In some embodiments, SEQ ID NO: 333 is inserted into SEQ ID NO: 1496. In some embodiments, SEQ ID NO: 417 is inserted into SEQ ID NO: 1496. In some embodiments, SEQ ID NO: 442 is inserted into SEQ ID NO: 1496. In some embodiments, SEQ ID NO: 673 is inserted into SEQ ID NO: 1496. SEQ ID NO: 1496 is AAV2 with an amino acid substitution. In some embodiments, any of the CNS-targeting molecules set forth in any of SEQ ID NO: 263-1495 is inserted into AAV9. In some embodiments, AAV9 comprises the sequence set forth in SEQ ID NO: 1497. In some embodiments, SEQ ID NO: 333 is inserted into SEQ ID NO: 1497. In some embodiments, SEQ ID NO: 417 is inserted into SEQ ID NO: 1497. In some embodiments, SEQ ID NO: 442 is inserted into SEQ ID NO: 1497. In some embodiments, SEQ ID NO: 673 is inserted into SEQ ID NO: 1497. SEQ ID NO: 1497 is AAV9. SEQ ID NO: 333 inserted into SEQ ID NO: 1497 can result in SEQ ID NOS: 1498, 1499, or 1500. In some embodiments, any of the CNS-targeting molecules set forth in any of SEQ ID NO: 263-1495 is inserted into an AAV. In some embodiments, the AAV capsid protein comprises the sequence set forth in SEQ ID NOS: 1498, 1499, or 1500.

[0114] In some embodiments, insertion of SEQ ID NO: 333 into AAV9 results in the sequence set forth in SEQ ID NO: 1498 (referred to as STAC-BBB; CNSRCV300). In some embodiments, insertion of SEQ ID NO: 333 into AAV9 results in a variant sequence of SEQ ID NO: 1496 or 1497. In embodiments, the variant comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 substitutions, insertions, and/or deletions of any of the amino acids in the amino acid sequence of SEQ ID NO: 1496 or 1497. In some embodiments, the variant comprises at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, or at least 99% sequence identity with SEQ ID NOS: 1498, 1499, or 1500 or comprises SEQ ID NOS: 1498, 1499, or 1500. In some embodiments, the variant comprises at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, or at least 99% sequence identity with SEQ ID NOS: 1498, 1499, or 1500 but is not SEQ ID NO: 1496 or 1497. In some embodiments, the variant comprises at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, or at least 99% sequence identity with SEQ ID NOS: 1498, 1499, or 1500 but is not a wild type AAV. In embodiments, the variant comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 1498, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 333. In embodiments, the variant comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 1498, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 417. In embodiments, the variant comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 1498, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 442. In embodiments, the variant comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 1498, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 673. In embodiments, the variant comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 1499, or 1500, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 333. In embodiments, the variant comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 1499, or 1500, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 417. In embodiments, the variant comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 1499, or 1500, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 442. In embodiments, the variant comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 1499, or 1500, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 673.

[0115] In some embodiments, the variant refers to a variant in the nucleotide sequence that encodes the amino acid sequences set forth in SEQ ID NOS: 1498, 1499, or 1500. In embodiments, the variant in the nucleotide sequence encodes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,

12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 substitutions, insertions, and/or deletions of any of the amino acids in the amino acid sequence of SEQ ID NOS: 1498, 1499, or 1500. In some embodiments, the variant in the nucleotide sequence encodes an amino acid sequence that comprises at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, or at least 99% sequence identity with SEQ ID NO: 1498 1498, 1499, or 1500. In embodiments, the variant in the nucleotide sequence encodes an amino acid sequence that comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NOS: 1498, 1499, or 1500, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 333. In embodiments, the variant in the nucleotide sequence encodes an amino acid sequence that comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NOS: 1498, 1499, or 1500, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 417. In embodiments, the variant in the nucleotide sequence encodes an amino acid sequence that comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NOS: 1498, 1499, or 1500, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 442. In embodiments, the variant in the nucleotide sequence encodes an amino acid sequence that comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NOS: 1498, 1499, or 1500, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 673.

[0116] Disclosed is a composition comprising, consisting, or consisting essentially of a) an AAV capsid protein comprising (i) a parent amino acid sequence selected from the group consisting of SEQ ID NO: 1496 or SEQ ID NO: 1497, and (ii) at least one targeting peptide inserted into the parent amino acid sequence of (i), wherein the targeting peptide is selected from any member of the group comprising, consisting, or consisting essentially of SEQ ID NO1: 263-1495 and b) expression construct encoding a fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene. In some embodiments, the ZFP domain comprises DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3. In some embodiments, the fusion protein comprises a ZFR shown in a single row of Tables 2 and 4.

[0117] Disclosed is a composition comprising, consisting, or consisting essentially of a) an AAV capsid protein comprising an amino acid sequence selected from the group comprising, consisting, or consisting essentially of SEQ ID NO: 1498, SEQ ID NO: 1499, or SEQ ID NO: 1500 and b) expression construct encoding a fusion protein comprising a zinc

finger protein (ZFP) domain and a transcription repressor domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene. In some embodiments, the ZFP domain comprises DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3. In some embodiments, the fusion protein comprises a ZFR shown in a single row of Tables 2 and 4.

[0118] AAV particles

[0119] In some embodiments, the CNS-targeting molecules are part of an engineered AAV capsid protein, and the engineered AAV capsid proteins are packaged into AAV particles. In some embodiments, the AAV particles that have enhanced tropism for a target tissue (*e.g.,* CNS and PNS) are provided. CNS-targeting molecules may be inserted into an AAV capsid protein sequence to alter tropism to a particular cell-type, tissue, organ or organism, *in vivo, ex vivo* or *in vitro*. In some embodiments, the AAV particles are capable of penetrating the blood brain barrier.

[0120] Delivery of AAV Particles

[0121] The AAV particles may be delivered to one or more target cells, tissues, organs, or organisms. In some embodiments, the AAV particles demonstrate enhanced tropism for a target cell type, tissue or organ. As a non-limiting example, the AAV particle may have enhanced tropism for cells and tissues of the central or peripheral nervous systems (CNS and PNS, respectively), or cells and tissues of a muscle. The AAV particles may, in addition, or alternatively, have decreased tropism for an undesired target cell-type, tissue or organ.

[0122] In some embodiments, the AAV particles are used to deliver a viral genome to a tissue or cells such as CNS or PNS cell or tissue.

[0123] The delivered viral genome may include genetic material of interest, such as, for example, an antibody, an enzyme, or regulatory RNA, amongst others. In some embodiments, the viral genome includes at least one ITR sequence. In some embodiments, the viral genome includes 2 ITR sequences. In some embodiments, the ITR sequences flank the genetic material of interest. In some embodiments, the ITR sequences are complementary to each other. In some embodiments, the ITR regions are derived from the same serotype as the capsid protein. ITR regions may be between 100 and 150 nucleotides in length.

[0124] In some embodiments, the AAV particles can be used to infect a wide range of cells (including quiescent and dividing cells) without integration into the host genome and without replicating. In some embodiments, the genome of the virus contains the components required for the assembly of a functional recombinant virus, or viral particle, which is loaded

with or engineered to target a particular tissue and express or deliver genetic material of interest to the particular tissue.

[0125] AAV capsid proteins comprising CNS-targeting molecules (targeting peptides)

[0126] In some embodiments, the CNS-targeting molecules, *i.e.,* targeting peptides, are part of a recombinant AAV capsid protein. In some embodiments, AAV capsid proteins described herein may be produced recombinantly and may be based on adeno-associated virus (AAV) wild type sequence.

[0127] CNS-targeting molecules may be inserted into an AAV capsid protein sequence to alter tropism relative to the natural AAV capsid protein, to a particular cell-type, tissue, organ or organism, *in vivo*, *ex vivo* or *in vitro*. Stated another way, CNS-targeting molecules, which refer to the targeting peptides, that are inserted into the capsid protein, allow the capsid protein to penetrate the blood brain barrier.

[0128] In some embodiments, the targeting peptide is used for enhanced or improved transduction of a target cell or tissue (*e.g.,* cells or tissues of the central nervous system (CNS) or peripheral nervous system (PNS)). In some embodiments, the targeting peptide is used to facilitate the AAV capsid protein across the blood brain barrier following administration to a subject. In some embodiments, the targeting peptide is used for enhanced or improved distribution of the genetic material throughout the multiple brain regions, *e.g.,* frontal cortex, sensory cortex, motor cortex, putamen, thalamus, cerebellar cortex, dentate nucleus, caudate, and/or hippocampus. In some embodiments, the targeting peptide is used for enhanced or improved genetic material expression in multiple brain regions. In some embodiments, the targeting peptide is used for enhanced or improved delivery of genetic material of interest to a desired tissue, cell, or organelle.

[0129] In some embodiments, the targeting peptide increases tropism of the AAV capsid to a cell, region, or tissue of the CNS. Examples of CNS cells include but are not limited to neurons (*e.g.,* excitatory neurons, inhibitory neurons, and motor neurons) and glial cells (*e.g.,* ependymal cells, astrocytes, oligodendrocytes. Examples of CNS tissue include but are not limited to the cortex (*e.g.,* frontal cortex, parietal cortex, occipital cortex, temporal cortex), thalamus, hypothalamus, striatum, hippocampus, entorhinal cortex, and basal ganglia.

[0130] In some embodiments, the AAV capsid protein comprising a targeting peptide is capable of increased tropism by at least 1.1-, 1.2-, 1.3-, 1.4-, 1.5-fold, relative to an AAV capsid protein that lacks a targeting peptide. In some embodiments, the AAV capsid

protein comprising a targeting peptide is capable of increased tropism by over 1.5-fold, relative to an AAV capsid protein that lacks a targeting peptide.

[0131] In some embodiments, the AAV capsid protein comprising the targeting peptide facilitates increased expression of delivered genetic material (*e.g.,* a therapeutic cargo) by at least 1.1-, 1.2-, 1.3-, 1.4-, 1.5-fold in a specific cell, region, or tissue, relative to an AAV capsid protein that lacks a targeting peptide. In some embodiments, the AAV capsid protein comprising the targeting peptide facilitates increased expression of delivered genetic material (*e.g.,* a therapeutic cargo) by more than 1.5-fold in a specific cell, region, or tissue, relative to an AAV capsid protein that lacks a targeting peptide.

[0132] In some examples, the targeting peptide is between 6 amino acids and 20 amino acids in length, for example 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length. In some examples, the targeting peptide is between 9 and 16 amino acids in length. In some examples, the targeting peptide is 9 amino acids in length. In some examples, the targeting peptide is 16 amino acids in length.

[0133] In some embodiments, the targeting peptide comprises an amino acid sequence of any sequence set forth in SEQ ID NO: 263-1495. In some embodiments, the targeting peptide comprises the amino acid sequence set forth in SEQ ID NO: 333. In some embodiments, the targeting peptide comprises the amino acid sequence set forth in SEQ ID NO: 417. In some embodiments, the targeting peptide comprises the amino acid sequence set forth in SEQ ID NO: 442. In some embodiments, the targeting peptide comprises the amino acid sequence set forth in SEQ ID NO: 673. In some embodiments, the targeting peptide comprises at least 3, 4, 5, 6, 7, 8, 9, or all contiguous amino acids of any sequence set forth in SEQ ID NO: 263-1495. In some embodiments, the targeting peptide comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the sequence set forth in SEQ ID NO: 333. In some embodiments, the targeting peptide comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the sequence set forth in SEQ ID NO: 417. In some embodiments, the targeting peptide comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the sequence set forth in SEQ ID NO: 443. In some embodiments, the targeting peptide comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the sequence set forth in SEQ ID NO: 673. In some embodiments, the targeting peptide is part of an AAV vector. In some embodiments, the targeting peptide is part of a capsid protein of the AAV vector. In some embodiments, nucleic acid sequences encode targeting peptides.

[0134] In some embodiments, the AAV capsid protein comprise a nucleic acid sequence encoding a peptide that comprises an amino acid sequence of any sequence set forth

in SEQ ID NO: 263-1495. In some embodiments, the AAV capsid protein comprise a nucleic acid sequence encoding a peptide that comprises an amino acid sequence of SEQ ID NO: 333. In some embodiments, the AAV capsid protein comprise a nucleic acid sequence encoding a peptide that comprises an amino acid sequence of SEQ ID NO: 417. In some embodiments, the AAV capsid protein comprise a nucleic acid sequence encoding a peptide that comprises an amino acid sequence of SEQ ID NO: 442. In some embodiments, the AAV capsid protein comprise a nucleic acid sequence encoding a peptide that comprises an amino acid sequence of SEQ ID NO: 673. In some embodiments, the AAV capsid protein comprise a nucleic acid sequence encoding a peptide that comprises at least 3, 4, 5, 6, 7, 8, 9, or all contiguous amino acids of any sequence set forth in SEQ ID NO: 263-1495. In some embodiments, the AAV capsid protein comprise a nucleic acid sequence encoding a peptide that comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the sequence set forth in SEQ ID NO: 333. In some embodiments, the AAV capsid protein comprise a nucleic acid sequence encoding a peptide that comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the sequence set forth in SEQ ID NO: 417. In some embodiments, the AAV capsid protein comprise a nucleic acid sequence encoding a peptide that comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the sequence set forth in SEQ ID NO: 442. In some embodiments, the AAV capsid protein comprise a nucleic acid sequence encoding a peptide that comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the sequence set forth in SEQ ID NO: 673.

[0135] Insertion of Targeting Peptides into Capsid Proteins

[0136] In some embodiments, a targeting peptide is a part of a capsid protein, and the targeting peptide is inserted at a location between amino acid residues 450 and 600 of the capsid protein. In some embodiments, the amino acid sequence is inserted at a location between amino acid residues 587 and 590 of the AAV9 capsid protein. In some embodiments, the amino acid sequence is inserted at a location between amino acid residues 384 and 386 of the AAV9 capsid protein. In some embodiments, the amino acid sequence is inserted at location between amino acid residues 588 and 589 of the AAV2 capsid protein.

[0137] In some embodiments, a peptide sequence that comprises any of the sequences set forth in SEQ ID NO: 263-1495 is inserted into the capsid protein. In some embodiments, the peptide sequence comprises 3, 4, 5, 6, 7, 8, 9 or all contiguous amino acids of any of the sequences set forth in SEQ ID NO: 263-1495. In some embodiments, disclosed is a peptide sequence that comprises the sequence set forth in SEQ ID NOS: 333, 417, 442, or 673. In some embodiments, the peptide sequence comprises 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the sequence set forth in SEQ ID NOS: 333, 417, 442, or 673.

[0138] In some embodiments, a targeting peptide is inserted into any of the AAV capsid protein comprises any of the AAV serotypes AAV1, AAV2, AAV3B, AAV5, AAV6, AAV8 and AAV9. In some embodiments, the AAV capsid protein comprises the AAV2 serotype. In some embodiments, the AAV2 serotype includes a mutation at position 588 from arginine to alanine. In some embodiments, the AAV2 serotype comprises the sequence of SEQ ID NO: 1496. In some embodiments, an amino acid sequence is inserted between positions 450 and 600 of the AAV capsid protein. In some embodiments, an amino acid sequence is inserted between positions 450 and 600 of SEQ ID NO: 1496. In some embodiments, an amino acid sequence is inserted between positions 588 and 589 of the AAV capsid protein. In some embodiments, an amino acid sequence is inserted between positions 588 and 589 of SEQ ID NO: 1496. In some embodiments, the AAV capsid protein comprises the AAV9 serotype. In some embodiments, the AAV9 serotype includes the sequence of SEQ ID NO: 1497. In some embodiments, an amino acid sequence is inserted between positions 450 and 600 of the AAV capsid protein. In some embodiments, an amino acid sequence is inserted between positions 450 and 600 of SEQ ID NO: 1497. In some embodiments, the amino acid sequence is inserted between positions 587 and 590 of the AAV capsid protein. In some embodiments, the amino acid sequence is inserted between positions 587 and 590 of SEQ ID NO: 1497. In some embodiments, the amino acid sequence is inserted between positions 384 and 386 of the AAV capsid protein. In some embodiments, the amino acid sequence is inserted between positions 384 and 386 of SEQ ID NO: 1497. In some embodiments, the amino acid sequence inserted into the AAV2 or AAV9 serotypes comprises an amino acid sequence set forth in SEQ ID NO: 263-1495. In some embodiments, the amino acid sequence inserted into the AAV2 or AAV9 serotypes comprises an amino acid sequence set forth in SEQ ID NOS: 333, 417, 442, or 673. In some embodiments, insertion of the amino acid sequence set forth in SEQ ID NOS: 333 into an AAV9 serotype results in the sequence set forth in SEQ ID NO: 1498 (STAC-BBB, CNSRCV300), SEQ ID NO: 195 is the same as SEQ ID NO: 1498. In some embodiments, the AAV serotype comprises the sequence of SEQ ID NO: 1498. In some embodiments, the AAV serotype comprises the sequence of SEQ ID NOS: 1498, 1499, 1500 and combinations thereof. In some embodiments, insertion of the amino acid sequence set forth in SEQ ID NO: 333 into an AAV serotype results in the sequence set forth in SEQ ID NOS: 1498, 1499, or 1500.

[0139] In some embodiments, a targeting peptide is inserted into an AAV capsid protein. Any targeting peptide described herein may be inserted into a parent AAV capsid protein in any location that results in fully functional AAV particles. The targeting peptide may be inserted into capsid proteins VP1, VP2 and/or VP3. In some embodiments, a targeting

peptide, is inserted in a hypervariable region of the AAV capsid protein. Non-limiting examples of such hypervariable and/or surface exposed loop of the AAV capsid protein. In some embodiments, the targeting peptide is inserted into the H1 loop. In some embodiments, the targeting peptide is inserted into the DE loop. In some embodiments, the targeting sequencing is inserted into the variable region of the surface exposed loop, for example any of VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VIII and VR-IX. In some embodiments, the targeting peptide comprises any of SEQ ID NO: 263-1495. In some embodiments, the targeting peptide comprises SEQ ID NOS: 333, 417, 442, or 673.

[0140] In some embodiments, the AAV capsid proteins described herein have enhanced tropism for a specific cell or tissue, for example, a CNS or PNS cell or tissue. In some embodiments, the enhanced tropism for a specific cell or tissue is due to the insertion of any of the sequences set forth in SEQ ID NO: 263-1495 into the AAV capsid protein. In some embodiments, the enhanced tropism for a specific cell or tissue is due to the insertion of SEQ ID NO: 333, 417, 442, or 673 in to the AAV capsid protein. In some embodiments, the AAV capsid proteins are capable of penetrating the blood brain barrier. In some embodiments, the AAV capsid proteins described herein are capable of penetrating the blood brain barrier due to the insertion of any of the sequences set forth in SEQ ID NO: 263-1495 into the AAV capsid protein. In some embodiments, the AAV capsid proteins described herein are capable of penetrating the blood brain barrier due to the insertion of SEQ ID NO: 333, 417, 442, or 673 into the AAV capsid protein. In some embodiments, the AAV capsid proteins described herein are capable of distributing throughout multiple brain regions including, but not limited to the frontal cortex, sensory cortex, motor cortex, putamen, thalamus, cerebellar cortex, and hippocampus. In some embodiments, the AAV capsid proteins described herein are capable of distributing throughout multiple brain regions due to the insertion of any of the SEQ ID NO: 263-1495 into the AAV capsid protein. In some embodiments, the AAV capsid proteins described herein are capable of distributing throughout multiple brain regions due to the insertion of SEQ ID NO: 333, 417, 442, or 673 into the AAV capsid protein.

[0141] The peptide sequences listed in Table 6 were inserted into variable region 8 of AAV serotypes 2 and 9. In some embodiments, the peptides are inserted into AAV9 (SEQ ID 1497) between 587/590, the insertions are after amino acid 587 and before 590 replacing amino acids 588 and 589. Other peptides inserted into AAV9 indicated in Table 6 are inserted between amino acids 588/589. Peptides inserted into AAV2, were inserted between positions 588/589 and the wildtype arginine at position 588 is altered to alanine (R588A) (Seq ID 1496). For more detail see WO2024238684, incorporated by reference in its entirety.

Table 6.

Sequence ID	Peptide Sequence
Seq ID 263	NFRQSEMLN
Seq ID 264	QRNTDHITP
Seq ID 265	MQFDPQKPD
Seq ID 266	TMSSNDNMR
Seq ID 267	VKESVQKAA
Seq ID 268	ILNVSKPDN
Seq ID 269	SQAAQLPTQ
Seq ID 270	QVMAIDAAI
Seq ID 271	NDTARERAI
Seq ID 272	SMAAKVTAE
Seq ID 273	TLGHQHSPI
Seq ID 274	TVPSSGKQH
Seq ID 275	AMVQISTPL
Seq ID 276	VQVTGTVRF
Seq ID 277	MIAQKTVVH
Seq ID 278	NTHFQGQPP
Seq ID 279	MDMQATGKN
Seq ID 280	NLAHTRQPQ
Seq ID 281	LDIPPMNSS
Seq ID 282	QHSTPSWPS
Seq ID 283	AFTRHNGTGGKSIYGA
Seq ID 284	ARGARDEFT
Seq ID 285	RPSNDKAMM
Seq ID 286	AQVHMTMPQ
Seq ID 287	ARQTFQHHL
Seq ID 288	HQTDAPTDW
Seq ID 289	ANMISEPRISYGNDAA
Seq ID 290	IERWQAEII
Seq ID 291	VPHNGLHST
Seq ID 292	RIHDPSLVS
Seq ID 293	IPATHPQLM
Seq ID 294	ITTVKNVTV
Seq ID 295	DKSVKSTAI
Seq ID 296	IRTHEVPLQ
Seq ID 297	SPSSTSNNN
Seq ID 298	LSTTSQVHN
Seq ID 299	NMHNETSMT
Seq ID 300	NDSRKANIG
Seq ID 301	KPPNVQDAK
Seq ID 302	FQHMTPKPS
Seq ID 303	KQPSRLMEW
Seq ID 304	VYDQVKATH
Seq ID 305	SYRNQYDDQ

Sequence ID	Peptide Sequence
Seq ID 878	MKVSAQVQS
Seq ID 879	NVSQQKMVQ
Seq ID 880	RQQNQDKFS
Seq ID 881	MSSRTMEIH
Seq ID 882	HEMIKQSGP
Seq ID 883	QDTDRAGKF
Seq ID 884	KWEQRGMAH
Seq ID 885	TKQHTEMQQ
Seq ID 886	PMQALQNML
Seq ID 887	KVAFQQQIE
Seq ID 888	QVPRQEVNE
Seq ID 889	HLEREHKWD
Seq ID 890	IQRKIQDLA
Seq ID 891	IKGTDSMQQ
Seq ID 892	TMVSGEARQ
Seq ID 893	TMVASRQPP
Seq ID 894	QQRQHQEIT
Seq ID 895	NPESMEAYI
Seq ID 896	TSQNGKMSV
Seq ID 897	SRTSTQPQE
Seq ID 898	RYAVKGETD
Seq ID 899	VALYPNHAE
Seq ID 900	QQTTQHIDS
Seq ID 901	SSMQEASQI
Seq ID 902	KMNSPNQER
Seq ID 903	MTNRTPASN
Seq ID 904	IGVRPMISP
Seq ID 905	RQADSRTIE
Seq ID 906	EMRNHPAVQ
Seq ID 907	MRTNEPHKE
Seq ID 908	PTIQRQGMN
Seq ID 909	DIDPEDIST
Seq ID 910	EIKMQSTQA
Seq ID 911	QNFPSQNAA
Seq ID 912	YRTGPDEIQ
Seq ID 913	IQHTHSHHE
Seq ID 914	IISTQEGHI
Seq ID 915	PEGDNTFMI
Seq ID 916	RANAEYLSD
Seq ID 917	EVSLPSNVK
Seq ID 918	RMDINRATD
Seq ID 919	HPSPNTAYV
Seq ID 920	SYHSWFPMG
Seq ID 921	AGDRQHMAA

Seq ID 306	VQSTKQSVQ
Seq ID 307	HQGKVDAQN
Seq ID 308	IQGEIRVHT
Seq ID 309	TQTLKVEDR
Seq ID 310	VQSPPGIMP
Seq ID 311	VHYAPNYTT
Seq ID 312	YGGSETNVR
Seq ID 313	IRTQTHPQV
Seq ID 314	ETQVQPIQH
Seq ID 315	FPVSSVNDV
Seq ID 316	IQKISQAMA
Seq ID 317	SQTQIGQPY
Seq ID 318	AIRTNVGPV
Seq ID 319	TQMSGQTPH
Seq ID 320	PAKSVMIPY
Seq ID 321	QRVSQTNSF
Seq ID 322	AFAGANYAAWAVNVAA
Seq ID 323	AVVRPQSTM
Seq ID 324	PLQTPPSDN
Seq ID 325	VTVSQQMTK
Seq ID 326	DPFEGRIST
Seq ID 327	NNPTGGIAL
Seq ID 328	ARYTHLVAIQNKEEIA
Seq ID 329	IVTGGVLKP
Seq ID 330	THMSTQASI
Seq ID 331	AVPGEEEQKFEVEKYA
Seq ID 332	ATDTSHLVV
Seq ID 333	YVNIMDDMD
Seq ID 334	IRPDKQGGV
Seq ID 335	NQTESQWKG
Seq ID 336	TNEGLYKDN
Seq ID 337	IMVGNQTVM
Seq ID 338	IWAEKGLDT
Seq ID 339	MPSQGYQQV
Seq ID 340	NTFGHNVAP
Seq ID 341	ASRTVSDSIKKLITSA
Seq ID 342	NHTSTPPQQ
Seq ID 343	NAHAPIPVE
Seq ID 344	KSTNTHEIG
Seq ID 345	NTRVTNAHD
Seq ID 346	AHRDACLTGSKIQVDA
Seq ID 347	AVAAETGQI
Seq ID 348	NQPSMVNGH
Seq ID 349	PMTQQHHSM
Seq ID 350	YEKVDSLMM

Seq ID 922	QPKKWGADY
Seq ID 923	LKQDSRPTY
Seq ID 924	VSSYQLKSD
Seq ID 925	RGHSVSDNA
Seq ID 926	HQAQNQMFK
Seq ID 927	QASNFITVP
Seq ID 928	VEPHYQQQW
Seq ID 929	TQGRSQEAT
Seq ID 930	ATSSRTTLA
Seq ID 931	PSSIGKHDN
Seq ID 932	RHSGDSIPE
Seq ID 933	ARPATTQSN
Seq ID 934	PAPSGNTMA
Seq ID 935	KSPSMSVVL
Seq ID 936	IPGPNGQIP
Seq ID 937	PQDYHMSEH
Seq ID 938	ATHLRVIYERMNQSLA
Seq ID 939	AHGKREVALHLHPDHA
Seq ID 940	PHTHAQMNL
Seq ID 941	TVPSQQPGT
Seq ID 942	AEYKDCHLAQVPSHTA
Seq ID 943	AVGALIQRGFIRPKDA
Seq ID 944	TETDPMQRM
Seq ID 945	HRQVAGEST
Seq ID 946	SQSPTRIIQ
Seq ID 947	RLAIPDGIT
Seq ID 948	RPPQNPQTI
Seq ID 949	VELMRTHST
Seq ID 950	HQPQYSKSD
Seq ID 951	ATMQCAHTA
Seq ID 952	KMGGVAAKI
Seq ID 953	TMNKSADRG
Seq ID 954	EVDDFVSYG
Seq ID 955	PMSQKVQSA
Seq ID 956	IHSKANDVL
Seq ID 957	AVVSPANAA
Seq ID 958	RQTQTASEY
Seq ID 959	MESHPQAPQ
Seq ID 960	TFRESEMIM
Seq ID 961	HHTPQNMVN
Seq ID 962	KTQQMTPVT
Seq ID 963	IAPQQTYWK
Seq ID 964	EVHSKVMHP
Seq ID 965	NKSTSTVAW
Seq ID 966	ADSAQAMTK
Seq ID 967	MESRKPNDI

Seq ID 351	MDVGTHGKA	Seq ID 968	NVAQTMIRV
Seq ID 352	THNSQVHPS	Seq ID 969	HPRDHEHVD
Seq ID 353	PTQVRQVQQ	Seq ID 970	RVNGSQKES
Seq ID 354	ITRTVNTVE	Seq ID 971	YSRASSETA
Seq ID 355	AMIMSPFGT	Seq ID 972	NWGQTQAKQ
Seq ID 356	HAFPSFPAA	Seq ID 973	AQEMSKDLEEVKAKVA
Seq ID 357	KMMDNTPFY	Seq ID 974	REMRASQVE
Seq ID 358	PQRLSVDPN	Seq ID 975	QYIQEHVAK
Seq ID 359	TWGTYQHSV	Seq ID 976	QVAQTTLPI
Seq ID 360	HYQQRDMSQ	Seq ID 977	NTVQNMAHQ
Seq ID 361	SQGHVQERM	Seq ID 978	TQSREMDTQ
Seq ID 362	QPRTTGNDF	Seq ID 979	DDAAHHGFD
Seq ID 363	PKGTISSQQ	Seq ID 980	AATPSPVLPDIVMEAA
Seq ID 364	REHTAAWFA	Seq ID 981	QQASGNNLR
Seq ID 365	QLTHSNTSQ	Seq ID 982	AKVVTDSMS
Seq ID 366	KDMGAGHSM	Seq ID 983	SQVVSQGSQ
Seq ID 367	ARGAQRLAALGDTAWA	Seq ID 984	FQHRANSME
Seq ID 368	KQAHREHEE	Seq ID 985	ANRGTEWDA
Seq ID 369	KSSHPYTDE	Seq ID 986	TIDEVDIGY
Seq ID 370	MPNHVAYKD	Seq ID 987	LQYKAMQVS
Seq ID 371	PPTTQYPQT	Seq ID 988	AIPVCEAFQCTALSNA
Seq ID 372	RSHTTGQDS	Seq ID 989	TEDSPQMTQ
Seq ID 373	SQLKASVTN	Seq ID 990	SGSMTPTTV
Seq ID 374	TAPSTRFEV	Seq ID 991	LPMPTSQGI
Seq ID 375	PSTLEPEVW	Seq ID 992	SYPTPQSQT
Seq ID 376	YTSPPQMMV	Seq ID 993	SASKSFPTA
Seq ID 377	PVPSRQVST	Seq ID 994	PSQPMKFIN
Seq ID 378	LEKVQVHQN	Seq ID 995	VEAQHKPAY
Seq ID 379	NQIKLQSMS	Seq ID 996	IPSPVTAYW
Seq ID 380	GVMSPTPMV	Seq ID 997	NYLSTSVPR
Seq ID 381	PTQRAERYF	Seq ID 998	TNSVKMPPS
Seq ID 382	PTAKVVDSV	Seq ID 999	PQEDVKSVM
Seq ID 383	HRIHEVQGG	Seq ID 1000	EPHNMKVEK
Seq ID 384	KNTTPVGQQ	Seq ID 1001	PQKQSLHTV
Seq ID 385	AGPPLKGAGGKERPGA	Seq ID 1002	KPQGVNVTY
Seq ID 386	IQPASGQNM	Seq ID 1003	FQRSEVQTI
Seq ID 387	YQNQGVNVP	Seq ID 1004	AAEPAPDFSDYSEMAA
Seq ID 388	NASSQSNAN	Seq ID 1005	QRQGGMEAK
Seq ID 389	SNTVRGDAA	Seq ID 1006	TASVKQQNT
Seq ID 390	SKKAEFGTE	Seq ID 1007	HRNTESGDQ
Seq ID 391	KVSSEMMVH	Seq ID 1008	FPKSYEQVQ
Seq ID 392	TMIEQRMDQ	Seq ID 1009	QGASQRVAI
Seq ID 393	PPHIYHHMS	Seq ID 1010	HPMAKDVVK
Seq ID 394	MVNKAPTGA	Seq ID 1011	REQKLYKEP
Seq ID 395	KSPVARGDT	Seq ID 1012	TEQQWYGRM
Seq ID 396	LLKQRQLMD	Seq ID 1013	APTNKTVGI

Seq ID 397	KMKQIELVE	Seq ID 1014	QRTAVNEMQ
Seq ID 398	RAPNRVNDE	Seq ID 1015	PQNAQRVDT
Seq ID 399	TMNQAPPAQ	Seq ID 1016	KDDDKVGIV
Seq ID 400	LVKEDEHWI	Seq ID 1017	APAPAAPPSTPAAPKA
Seq ID 401	MIGQRDNNS	Seq ID 1018	NHAVMHTVS
Seq ID 402	TSHMVSHAV	Seq ID 1019	MRTQAVDGF
Seq ID 403	IQDEEAKYT	Seq ID 1020	DIQHATKRE
Seq ID 404	TGGSKFPYM	Seq ID 1021	ATQAHTMMY
Seq ID 405	VEGSAQMIK	Seq ID 1022	QGTSEASRY
Seq ID 406	FTDISKEPS	Seq ID 1023	RNSHGMSES
Seq ID 407	TERPMMQQL	Seq ID 1024	PNSAHNNSY
Seq ID 408	VSLGHDKPV	Seq ID 1025	TTGASKDKV
Seq ID 409	TQRTHISDM	Seq ID 1026	DRTGVWAGE
Seq ID 410	TSYSTHASS	Seq ID 1027	VQTLDRGSQ
Seq ID 411	NTKSADHEY	Seq ID 1028	DATAHSLIQ
Seq ID 412	RDPLREQRP	Seq ID 1029	IGQAPEVND
Seq ID 413	LGQRTVQAQ	Seq ID 1030	HYAKVTEQA
Seq ID 414	VHDMTHQVN	Seq ID 1031	DPSVRSNMI
Seq ID 415	DYPEYHDIW	Seq ID 1032	MSKVSTQDR
Seq ID 416	QTQQGNYPK	Seq ID 1033	AQDRISQWA
Seq ID 417	QASVGTVRV	Seq ID 1034	AHLAQPATLSQTIVPA
Seq ID 418	SPAHQRGVD	Seq ID 1035	NEKIRELPK
Seq ID 419	QMYGEKSVS	Seq ID 1036	QMTKHETYI
Seq ID 420	NTAVQRGSV	Seq ID 1037	GAQQYSQVS
Seq ID 421	KTIQQTQDV	Seq ID 1038	IQDDPMTSM
Seq ID 422	QMQTLGDDI	Seq ID 1039	VPTVERNSL
Seq ID 423	SHTGQTREL	Seq ID 1040	KLVMTQTEY
Seq ID 424	KRQNTESAF	Seq ID 1041	GGVPMKALI
Seq ID 425	NFDPLSVAE	Seq ID 1042	IQSPQQAMR
Seq ID 426	KILQQEPLM	Seq ID 1043	SFHGPIKNY
Seq ID 427	IVHAVIQST	Seq ID 1044	NTKDNAGKD
Seq ID 428	TMDHNPIQT	Seq ID 1045	ADFSDLYYHTMNNKHA
Seq ID 429	KQPTPTNLW	Seq ID 1046	QKQIMHPES
Seq ID 430	EQGNPGVSI	Seq ID 1047	PQPQMGHSM
Seq ID 431	IHSRVQQVT	Seq ID 1054	QLHTGIQNS
Seq ID 432	PQSQYRPAN	Seq ID 1055	AFEVMEDHAGTYGLGA
Seq ID 433	YRRDQENAI	Seq ID 1056	SYVQPTAIM
Seq ID 434	TANDAIVAT	Seq ID 1057	IQATRNVGV
Seq ID 435	ALYVGSKTKEGVVHGA	Seq ID 1058	MILVVLQSV
Seq ID 436	MASTNVGTS	Seq ID 1059	KMFSSSEQF
Seq ID 437	AIQASDTYI	Seq ID 1060	TTDRDKMAL
Seq ID 438	NMQPRIVNA	Seq ID 1061	KAINTMIEA
Seq ID 439	QSDKEQFKH	Seq ID 1062	HMTNGTPMM
Seq ID 440	SVINQQAKA	Seq ID 1063	MQNSVKQVW
Seq ID 441	NVPGSLKIQ	Seq ID 1064	SQPKMQETG
Seq ID 442	RKDNIPMMV	Seq ID 1065	TESHSITPM

Seq ID 443	NKVHGTIQD	Seq ID 1066	QVSQGVHGQ
Seq ID 444	SRKDKSEAE	Seq ID 1067	TTQMQPMVA
Seq ID 445	HAQASQTQF	Seq ID 1068	KLTVTRRMF
Seq ID 446	HLGHHDGAA	Seq ID 1069	AGIATSGSA
Seq ID 447	QPQMGVMVQ	Seq ID 1070	TPNMQSMSY
Seq ID 448	ANLVRNIDA	Seq ID 1071	FNDQPQKIF
Seq ID 449	SDIKKRNAV	Seq ID 1072	PTSVHGQML
Seq ID 450	RVMTNSIDV	Seq ID 1073	SQTHQVGYP
Seq ID 451	KHQQQLAEK	Seq ID 1074	QGTTATRPD
Seq ID 452	QQTPQTHPV	Seq ID 1075	AGGKVQIINKKLDLSA
Seq ID 453	IHNAHVPHQ	Seq ID 1076	ASLSITEDA
Seq ID 454	VSRTPHTDF	Seq ID 1077	TSKPPALQG
Seq ID 455	MQMKGQQPP	Seq ID 1078	KVMEKQGMR
Seq ID 456	HQSGQVPNI	Seq ID 1079	KTVESPQQQ
Seq ID 457	QLTGVASGH	Seq ID 1080	AGPSVGRAKGQDAPLA
Seq ID 458	PEQSPQMMF	Seq ID 1081	RHPSPVNVL
Seq ID 459	NTTPQASLM	Seq ID 1082	YQNDMRKGT
Seq ID 460	DEFGAYMPI	Seq ID 1083	LINAHQPQK
Seq ID 461	PKLIQQVNT	Seq ID 1084	AGHAIENAVRSSLKGA
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Seq ID 801	ENSRTTTVG	Seq ID 1417	GDTQPRMQY
Seq ID 802	TFVVGQSGK	Seq ID 1418	EYPSEIVYS
Seq ID 803	APLRPQTGE	Seq ID 1419	TWQHTTETS
Seq ID 804	QTSSSTHHA	Seq ID 1420	TGGAYPVIV
Seq ID 805	KIAATALSS	Seq ID 1421	AGYGSSSRRAPQTGIA
Seq ID 806	PESGQSKMH	Seq ID 1422	AVADSRSPA
Seq ID 807	VISVGQMAK	Seq ID 1423	AGIVSWGRGCALKDKA
Seq ID 808	AERPQQGMH	Seq ID 1424	AWESDDFTA
Seq ID 809	TQQNQIIQR	Seq ID 1425	SVDQSHDPN
Seq ID 810	ARQAQEKFGKDKSPKA	Seq ID 1426	ARPNLPLPA

Seq ID 811	VTAVTTPSH	Seq ID 1427	ARPNLPLPA
Seq ID 812	SQVRGPETT	Seq ID 1428	TDLADSEWR
Seq ID 813	PLNHQQIQR	Seq ID 1429	AAFTTTLRGAQRLAAA
Seq ID 814	AVVPRPHEI	Seq ID 1430	VSNPQPPSR
Seq ID 815	TPNMEDNRQ	Seq ID 1431	APSLPTPPTREPKKVA
Seq ID 816	HRVTGVNDV	Seq ID 1432	PTHTQGRES
Seq ID 817	FTGNQGAQN	Seq ID 1433	AQNAKNGNLPEFGDAA
Seq ID 818	PRLAETSSQ	Seq ID 1434	KNAVMAPGN
Seq ID 819	SPRQSDPAH	Seq ID 1435	ALVPIATQTYEAWLGA
Seq ID 820	MNDDQQPPP	Seq ID 1436	IPMRIETDP
Seq ID 821	QMKPLPQAM	Seq ID 1437	QARDSPKGW
Seq ID 822	YSRHENISY	Seq ID 1438	AHRERMSQVMREWEEA
Seq ID 823	TEGERVGML	Seq ID 1439	SRVSQNEHP
Seq ID 824	NHVPVLWAD	Seq ID 1440	QRNEKSEFI
Seq ID 825	TLAPLATTS	Seq ID 1441	QTLPEVTKW
Seq ID 826	NQRDVTNLT	Seq ID 1442	VKGWQVTER
Seq ID 827	QQNNTVPNQ	Seq ID 1443	APTTVESHGNYSTQIA
Seq ID 828	EKNMHTGVH	Seq ID 1444	PRQGPSTNL
Seq ID 829	VTLQGATPN	Seq ID 1445	AEATDGMLKIQFSAQA
Seq ID 830	PTKTSTGQM	Seq ID 1446	ALYRQKVEPLRAELQA
Seq ID 831	FNVDQHRNS	Seq ID 1447	RPLTTEAIV
Seq ID 832	PMTLLKDIQ	Seq ID 1448	KPGSQQIMD
Seq ID 833	DPTRPIETV	Seq ID 1449	AHQLLGDLHQEGPPLA
Seq ID 834	ERKQQLSEM	Seq ID 1450	MEQLVTFKT
Seq ID 835	NQEQFGMIV	Seq ID 1451	GASMQTALW
Seq ID 836	TTQIMVGTK	Seq ID 1452	AAFRCLAENAGDVAFA
Seq ID 837	MYAKTEVQW	Seq ID 1453	IQTQLPPGK
Seq ID 838	PVGHQMDVY	Seq ID 1454	NTVHQAQIM
Seq ID 839	QVFQDRTSL	Seq ID 1455	PISNVNQSR
Seq ID 840	LKDNQQATF	Seq ID 1456	APDLVQEACESELNEA
Seq ID 841	AGNTHTLIN	Seq ID 1457	AKIQVLVEPDHFKVAA
Seq ID 842	KVPPQHLTA	Seq ID 1458	AREWFMDLNLPWSSAA
Seq ID 843	GIVTATRVQ	Seq ID 1459	AEFSSNTVKLTSGHLA
Seq ID 844	NIAHHQGSN	Seq ID 1460	AFTYKSSCAFSCEEGA
Seq ID 845	PAMPGAAAR	Seq ID 1461	PYPTQASMK
Seq ID 846	VQSITPPQS	Seq ID 1462	KPTNSGQEH
Seq ID 847	QATHQGQNH	Seq ID 1463	LSTGPPEKI
Seq ID 848	PTNAPGVTE	Seq ID 1464	APQLHVMSA
Seq ID 849	MHRAQAIAE	Seq ID 1465	TASKMQDSK
Seq ID 850	AQASEKMMK	Seq ID 1466	AFTSSSGQKAMNGSRA
Seq ID 851	RMNIPPNGT	Seq ID 1467	AVAEFSEATAELTVSA
Seq ID 852	RPSDINSGD	Seq ID 1468	INQNKAHDA
Seq ID 853	MQPKSAQNV	Seq ID 1469	AARLSKELQAAQARLA
Seq ID 854	LAASMQGAH	Seq ID 1470	ATKPGYVDSIQKGIQA
Seq ID 855	TSIITQAAN	Seq ID 1471	PTTAPSMVL
Seq ID 856	SDREETFNM	Seq ID 1472	AGGFVYIAGKCGLVPA

Seq ID 857	NQYNMQPSK
Seq ID 858	WVSEEHFLT
Seq ID 859	QSATSVTSQ
Seq ID 860	HARQSYNDP
Seq ID 861	AMNVISHDGTPA
Seq ID 862	STIMSEAEF
Seq ID 863	HANTNVLEK
Seq ID 864	KPQVTPAVH
Seq ID 865	AMPDRTMMR
Seq ID 866	VAIGQTKTL
Seq ID 867	PTDYSVKSV
Seq ID 868	IGHHKEMKE
Seq ID 869	ASQAIHYNA
Seq ID 870	QVRMEGAEY
Seq ID 871	MKERHEHFE
Seq ID 872	GQVLMAGTR
Seq ID 873	TVSMPVSHA
Seq ID 874	NHDRVNVQP
Seq ID 875	EELGILNLQ
Seq ID 876	ANGNFQVLK
Seq ID 877	RQYNTESMH

Seq ID 1473	ANPTAKRQFVQSAKEA
Seq ID 1474	ALALGRFWDYLRWVQA
Seq ID 1475	ALRVIYERMNQSLSLA
Seq ID 1476	VTMTQATNR
Seq ID 1477	ALLQYNHRVKKLNEIA
Seq ID 1478	ALLGKINLKALAALAA
Seq ID 1479	RQRVSYMDD
Seq ID 1480	ADMGQQRYD
Seq ID 1481	ATGVLLRALAGVPVAA
Seq ID 1482	SIKKDPPTV
Seq ID 1483	PGKTMLGNH
Seq ID 1484	HQGDTLQYV
Seq ID 1485	ALGLGKHNYCRNPDGA
Seq ID 1486	QERQANKSY
Seq ID 1487	QQTRGVELN
Seq ID 1488	ASMRVVPMA
Seq ID 1489	ASSTDKRDA
Seq ID 1490	ALIGGSIVA
Seq ID 1491	LLHTGTSSH
Seq ID 1492	TQHQAQVPR
Seq ID 1493	AAGLFGAAEGQAFHLA
Seq ID 1494	AENEHAHFQKAKERLA
Seq ID 1495	QALVPIATQTYEAWLGA

[0142] Seq ID 1496: AAV2 R588A

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYK
YLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQ
ERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEP
DSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNTMAT
GSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNH
LYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFR
PKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQG
CLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFS
YTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQFSQA
GASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSL
VNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTN
PVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWA
KIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYS
TGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPI

GTRYLTRNL

[0143] Seq ID 1497: AAV9

MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGY
KYLGPGNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEF
QERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQE
PDSSAGIGKSGAQPAKKRLNFGQTGDTESVPDPQPIGEPPAAPSGVGSLTMAS
GGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVITTSTRTWALPTYNNH
LYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWG
FRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSA
HEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNF
QFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFS
VAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRN
SLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIK
TTNPVATESYGQVATNHQSAQAQAQTGWVQNQGILPGMVWQDRDVYLQGP
IWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDKLNSFI
TQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGVYSE
PRPIGTRYLTRNL

[0144] Seq ID 1498: CNSRCV300 (STAC-BBB)

MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGY
KYLGPGNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEF
QERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQE
PDSSAGIGKSGAQPAKKRLNFGQTGDTESVPDPQPIGEPPAAPSGVGSLTMAS
GGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVITTSTRTWALPTYNNH
LYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWG
FRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSA
HEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNF
QFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFS
VAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRN
SLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIK
TTNPVATESYGQVATNHQSAYVNIMDDMDQAQTGWVQNQGILPGMVWQD
RDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAF
NKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFA
VNTEGVYSEPRPIGTRYLTRNL

[0145] STAC-BBB VP2 capsid amino acid sequence

[0146] KTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTES VPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLG DRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHF SPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSD YQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLR TGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFS VAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMN PGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATES YGQVATNHQSAYVNIMDDMDQAQTGWVQNQGILPGMVWQDRDVYLQGPIWAKIP HTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSV EIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL (SEQ ID NO: 1499)

[0147] STAC-BBB VP3 capsid amino acid sequence MASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHL YKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKR LNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPA DVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSY AHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGP SYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPL SGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAYVNIMD DMDQAQTGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGM KHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPE IQYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL (SEQ ID NO: 1500)

V. Pharmaceutical Applications

[0148] Zinc finger protein transcription factors for repressing PrP expression is disclosed in International Patent Publication WO 2021/067864, the entirety of which is incorporated by reference herein and specifically incorporated by reference in its entirety is the section related to Pharmaceutical Applications.

[0149] The ZFRs can be used to treat patients in need of downregulation of PrP expression. The patients suffer from, or are at risk of developing, prion disease. The prion disease to be treated may be familial, sporadic, or acquired prion disease, and may be CJD, sCJD, vCJD, GSS, FFI, sFI, Kuru, VPSPr. Patients at risk include those who are genetically predisposed, those who have been exposed to meat from cattle with mad cow disease, those

who have had been exposed due to iatrogenic means, or exposed to other environmental sources of prions. The disclosure provides a method of treating a neurodegenerative disease in a subject such as a human patient in need thereof, comprising introducing to the nervous system of the subject a therapeutically effective amount (*e.g.*, an amount that allows sufficient repression of *PRNP* expression) of the ZFR (*e.g.*, a rAAV vector expressing it). In some embodiments, the neurodegenerative disease is prion disease. The term "treating" encompasses any one of alleviation of symptoms, prevention of onset of symptoms, slowing of disease progression, and extension of survival. Biomarkers including, without limitation, prion, PrP, GFAP, or neurofilament light chain (NfL) levels in the cerebrospinal fluid or plasma may also be measured to monitor progress of the treatment.

[0150] The disclosure provides a pharmaceutical composition comprising a viral vector such as a recombinant rAAV whose recombinant genome comprises an expression cassette for the ZFRs. The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier such as water, saline (*e.g.*, phosphate-buffered saline), dextrose, glycerol, sucrose, lactose, gelatin, dextran, albumin, or pectin. In addition, the composition may contain auxiliary substances, such as, wetting or emulsifying agents, pH-buffering agents, stabilizing agents, or other reagents that enhance the effectiveness of the pharmaceutical composition. The pharmaceutical composition may contain delivery vehicles such as liposomes, nanocapsules, microparticles, microspheres, lipid particles, and vesicles.

[0151] In some embodiments, the pharmaceutical composition comprises STAC-BBB and a sequence encoding a prion-targeting transcriptional repressor (*e.g.*, ZFR). In some embodiments, the pharmaceutical composition comprises STAC-BBB and a vector encoding any one of the ZFP or ZFR sequences as shown in a single row in Tables 1-4.

[0152] The cells targeted by the therapeutics of the present disclosure are cells in the brain, including, without limitation, a neuronal cell (*e.g.*, a motor neuron, a sensory neuron, a dopaminergic neuron, a cholinergic neuron, a glutamatergic neuron, a GABAergic neuron, or a serotonergic neuron); a glial cell (*e.g.*, an oligodendrocyte, an astrocyte, a pericyte, a Schwann cell, or a microglial cell); an ependymal cell; or a neuroepithelial cell. The brain regions targeted by the therapeutics may be, for example, cerebral cortex (classic CJD), thalamus (FFI), brainstem (scrapie, BSE, and Chronic Wasting Disease), and cerebellum (Kuru). The targeted brain regions can be reached directly through intrastriatal injection, intrathalamic injection, intracerebral injection, intra-cisterna magna (ICM) injection, or more generally through intraparenchymal injection, intracerebroventricular (ICV) injection, intrathecal injection, or intravenous injection. Other routes of administration include, without

limitation, intracerebral, intraventricular, intranasal, or intraocular administration. In some embodiments, the viral vector spreads throughout the CNS tissue following direct administration into the cerebrospinal fluid (CSF), *e.g.*, via intrathecal and/or intracerebral injection, or intracisterna-magna injection. In other embodiments, the viral vectors cross the blood-brain-barrier and achieve wide-spread distribution throughout the CNS tissue of a subject following intravenous administration. In other embodiments, the viral vectors are delivered directly to the target regions via intraparenchymal injections. In some cases, the viral vectors may undergo retrograde or anterograde transport to other brain regions following intraparenchymal delivery. In some aspects, the viral vectors have distinct CNS tissue targeting capabilities (*e.g.*, CNS tissue tropisms), which achieve stable and nontoxic gene transfer at high efficiencies.

[0153] By way of example, the pharmaceutical composition may be provided to the patient through intraventricular administration, *e.g.*, into a ventricular region of the forebrain of the patient such as the right lateral ventricle, the left lateral ventricle, the third ventricle, or the fourth ventricle. The pharmaceutical composition may be provided to the patient through intracerebral administration, *e.g.*, injection of the composition into or near the striatum, caudate, putamen, substantia nigra, midbrain, olfactory bulb, cerebrum, medullar, pons, cerebellum, locus coeruleus, pons, medulla, brainstem, globus pallidus, hippocampus, cerebral cortex, cerebrum, intracranial cavity, meninges, dura mater, arachnoid mater, or pia mater of the brain. Intracerebral administration may include, in some cases, administration of an agent into the cerebrospinal fluid (CSF) of the subarachnoid space surrounding the brain.

[0154] In some cases, intracerebral administration involves injection using stereotaxic procedures. Stereotaxic procedures are well known in the art and typically involve the use of a computer and a 3-dimensional scanning device that are used together to guide injection to a particular intracerebral region, *e.g.*, a ventricular region. Micro-injection pumps (*e.g.*, from World Precision Instruments) may also be used. In some cases, a microinjection pump is used to deliver a composition comprising a viral vector. In some cases, the infusion rate of the composition is in a range of 0.1 µl/min to 100 µl/min. As will be appreciated by the skilled artisan, infusion rates will depend on a variety of factors, including, for example, species of the subject, age of the subject, weight/size of the subject, serotype of the AAV, dosage required, and intracerebral region targeted. Thus, other infusion rates may be deemed by a skilled artisan to be appropriate in certain circumstances.

[0155] Delivery of rAAVs to a subject may be accomplished, for example, by intravenous administration. In certain instances, it may be desirable to deliver the rAAVs

locally to the brain tissue, the spinal cord, cerebrospinal fluid (CSF), neuronal cells, glial cells, meninges, astrocytes, oligodendrocytes, microglia, interstitial spaces, and the like. In some cases, recombinant AAVs may be delivered directly to the CNS by injection into the ventricular region, as well as to the parenchyma, striatum, substantia nigra, cortex, cerebellar lobule, thalamus, hippocampus or other brain region or combination of brain regions. AAVs may be delivered with a needle, catheter or related device, using neurosurgical techniques known in the art, such as by stereotactic injection (*see, e.g.*, Stein et al., *J Vir.* (1999) 73:3424-9; Davidson et al., *PNAS.* (2000) 97:3428-32; Davidson et al., *Nat Genet.* (1993) 3:219-223; and Alisky and Davidson, *Hum. Gene Ther.* (2000) 11:2315-29.

[0156] Unless otherwise defined herein, scientific and technical terms used in connection with the disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the disclosure. In case of conflict, the specification, including definitions, will control. Generally, nomenclature used in connection with, and techniques of neurology, medicine, medicinal and pharmaceutical chemistry, and cell biology described herein are those well-known and commonly used in the art. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Throughout this specification and embodiments, the words "have" and "comprise," or variations such as "has," "having," "comprises," or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. All publications and other references mentioned herein are incorporated by reference in their entirety. Although a number of documents are cited herein, this citation does not constitute an admission that any of these documents forms part of the common general knowledge in the art. As used herein, the term "approximately" or "about" as applied to one or more values of interest refers to a value that is similar to a stated reference value. In certain embodiments, the term refers to a range of values that fall within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context.

[0157] In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

[0158] **Exemplary Embodiments**

[0159] Non-limiting exemplary embodiments of the disclosure are described below.

[0160] Embodiment 1: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 1, wherein the ZFP ID is 81613.

[0161] Embodiment 2: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 1, wherein the ZFP ID is 81747.

[0162] Embodiment 3: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 1, wherein the ZFP ID is 89324.

[0163] Embodiment 4: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 1, wherein the ZFP ID is 89322.

[0164] Embodiment 5: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 1, wherein the ZFP ID is 91973.

[0165] Embodiment 6: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 1, wherein the ZFP ID is 89302.

[0166] Embodiment 7: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 1, wherein the ZFP ID is 89427.

[0167] Embodiment 8: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 1, wherein the ZFP ID is 89352.

[0168] Embodiment 9: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 1, wherein the ZFP ID is 89347.

[0169] Embodiment 10: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 3, wherein the ZFP ID is 81638.

[0170] Embodiment 11: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition

helix sequences corresponding to a ZFP ID as shown in a single row of Table 3, wherein the ZFP ID is 89891.

[0171] Embodiment 12: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 3, wherein the ZFP ID is 89920.

[0172] Embodiment 13: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 3, wherein the ZFP ID is 89943.

[0173] Embodiment 14: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 3, wherein the ZFP ID is 91806.

[0174] Embodiment 15: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 3, wherein the ZFP ID is 91852.

[0175] Embodiment 16: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 3, wherein the ZFP ID is 91853.

[0176] Embodiment 17: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set

forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 3, wherein the ZFP ID is 91870.

[0177] Embodiment 18: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 3, wherein the ZFP ID is 91888.

[0178] Embodiment 19: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 3, wherein the ZFP ID is 91975.

[0179] Embodiment 20: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 3, wherein the ZFP ID is 91999.

[0180] Embodiment 21: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences corresponding to a ZFP ID as shown in a single row of Table 3, wherein the ZFP ID is 92071.

[0181] Embodiment 22: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 2, wherein the ZFP ID is 81613.

[0182] Embodiment 23: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 2, wherein the ZFP ID is 81747.

[0183] Embodiment 24: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 2, wherein the ZFP ID is 89324.

[0184] Embodiment 25: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 2, wherein the ZFP ID is 89322.

[0185] Embodiment 26: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 2, wherein the ZFP ID is 91973.

[0186] Embodiment 27: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 2, wherein the ZFP ID is 89302.

[0187] Embodiment 28: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition

helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 2, wherein the ZFP ID is 89427.

[0188] Embodiment 29: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 2, wherein the ZFP ID is 89352.

[0189] Embodiment 30: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 2, wherein the ZFP ID is 89347.

[0190] Embodiment 31: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 4, wherein the ZFP ID is 81638.

[0191] Embodiment 32: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 4, wherein the ZFP ID is 89891.

[0192] Embodiment 33: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 4, wherein the ZFP ID is 89920.

[0193] Embodiment 34: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set

forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 4, wherein the ZFP ID is 89943.

[0194] Embodiment 35: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 4, wherein the ZFP ID is 91806.

[0195] Embodiment 36: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 4, wherein the ZFP ID is 91852.

[0196] Embodiment 37: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 4, wherein the ZFP ID is 91853.

[0197] Embodiment 38: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 4, wherein the ZFP ID is 91870.

[0198] Embodiment 39: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 4, wherein the ZFP ID is 91888.

[0199] Embodiment 40: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 4, wherein the ZFP ID is 91975.

[0200] Embodiment 41: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 4, wherein the ZFP ID is 91999.

[0201] Embodiment 42: A composition comprising an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495 and an expression construct encoding a ZFR fusion protein that binds to a target sequence and comprises the DNA-binding zinc finger recognition helix sequences and backbone mutation(s) corresponding to a ZFP ID as shown in a single row of Table 4, wherein the ZFP ID is 92071.

[0202] Embodiment 43: The composition of any one of embodiments 1-42, wherein the ZFR fusion protein comprises a transcription repressor domain or epigenetic silencing domain.

[0203] Embodiment 44: The composition of embodiment 43, wherein transcription repressor domain comprises a KRAB domain.

[0204] Embodiment 45: The composition of embodiment 44, wherein transcription repressor domain comprises SEQ ID NO: 261.

[0205] Embodiment 46: The composition of any one of embodiments 1-45, wherein the AAV capsid encapsulates the expression construct.

[0206] Embodiment 47: The composition of any one of embodiments 1-46, wherein the AAV capsid sequence is at least 80%, 85%, 90%, 95%, or 99% identical to SEQ ID NOS: 1498, 1499, 1500 and combinations thereof.

[0207] Embodiment 48: The composition of any one of embodiments 1-47, wherein the ZFP domain binds to a target region of a human *PRNP* gene.

[0208] Embodiment 49: The composition of any one of embodiments 1-48, wherein the ZFP domain binds to a target region of a human *PRNP* gene, wherein the target region is within 1.5 kb of a transcription start site (TSS) in the *PRNP* gene.

[0209] Embodiment 50: The composition of any one of embodiments 1-49, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the target region is within 1000 bps upstream of the TSS, and/or within 500 bps downstream of the TSS of the *PRNP* gene.

[0210] Embodiment 51: The composition of any one of embodiments 48-50, wherein the fusion protein represses expression of the *PRNP* gene by at least about 40%, 75%, 90%, 95%, or 99% compared to an untreated patient with no or minimal detectable off-target binding or activity.

[0211] Embodiment 52: The composition of any one of embodiments 48-51, wherein the DNA-binding zinc finger recognition helix is linked to the transcription repressor or epigenetic silencing domain through a peptide linker.

[0212] Embodiment 53: The composition of any one of embodiments 1-52, wherein the DNA-binding zinc finger recognition helix binds to binds to a Target Sequence as shown in a single row in Tables 1 and 3.

[0213] Embodiment 54: A composition comprising: 1) an AAV capsid protein comprising at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in one of SEQ ID NOs: 333, 417, 442, or 673; and 2) an expression construct encoding a fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene, wherein the ZFP domain of the fusion protein comprises four, five, or six zinc fingers; binds to a Target Sequence shown in Tables 1 and 3; comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 22-30, 31-39, 40-48, 49-57, 58-66, 67-73 ordered as shown in Table 1; a DNA-binding recognition helix sequence comprising SEQ ID NOS: 112-123, 124-135, 136-147, 148-159, 160-171, 172-181 ordered as shown in Table 3; comprises a ZFP transcription factor shown in Tables 2 and 4; comprises the DNA-binding recognition helix sequences linked as shown in Tables 2 and 4; and/or comprises an amino acid sequence selected from SEQ ID NOs: 74-82 in Table 2 or 183-194 in Table 4.

[0214] Embodiment 55: A composition comprising an AAV capsid polypeptide, comprising a peptide modification relative to the AAV9 capsid polypeptide set forth in SEQ ID NO: 1497, wherein: the peptide modification is in variable region 8 (VRVIII) and; the peptide modification comprises an 9 amino acid insertion relative to the AAV9 capsid

polypeptide set forth in SEQ ID NO: 1497, and comprises the sequence set forth in any one of SEQ ID Nos:263-1495; and the portion of the capsid polypeptide that is not the peptide modification comprises at least or about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 1497 and an expression construct encoding a fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the ZFP domain comprises DNA-binding recognition helix sequences as shown in a single row in Tables 1 and 3.

[0215] Embodiment 56: A pharmaceutical composition comprising the composition of any one of embodiments 1-55, and a pharmaceutically acceptable carrier.

[0216] Embodiment 57: A host cell comprising the composition of any one of embodiments 1-55.

[0217] Embodiment 58: A host cell comprising the composition of any one of embodiments 1-55 wherein the host cell is a human cell.

[0218] Embodiment 59: A host cell comprising the composition of any one of embodiments 1-55 wherein the host cell is a brain cell.

[0219] Embodiment 60: A host cell comprising the composition of any one of embodiments 1-55 wherein the host cell is a brain cell selected from the group comprising, consisting, or consisting essentially of a motor neuron, a sensory neuron, a dopaminergic neuron, a cholinergic neuron, a glutamatergic neuron, a GABAergic neuron, or a serotonergic neuron, a glial cell, optionally an oligodendrocyte, an astrocyte, a pericyte, a Schwann cell, or a microglial cell, an ependymal cell, a neuroepithelial cell, or combinations thereof.

[0220] Embodiment 61: A method of inhibiting expression of PrP in a human brain cell, comprising introducing into a cell a composition of any one of embodiments 1-55.

[0221] Embodiment 62: A method of inhibiting expression of PrP in a human brain cell, comprising introducing into a subject in need thereof a composition of any one of embodiments 1-55.

[0222] Embodiment 63: The method of embodiment 61-62 wherein the AAV capsid penetrates across the blood brain barrier.

[0223] Embodiment 64: The method of any one of embodiments 61-63 wherein the patient shows ZFP expression.

[0224] Embodiment 65 The method of any one of embodiments 61-64 wherein the patient shows ZFP expression in the brain and/or spinal cord.

[0225] Embodiment 66: The method of any one of embodiments 61-65 wherein the patient does not show ZFP expression outside the brain and/or spinal cord.

[0226] Embodiment 67: The method of any one of embodiments 61-66 wherein the patient shows ZFP expression in ChAT-positive motor neurons in the spinal cord.

[0227] Embodiment 68: The method of any one of embodiments 61-67 wherein the patient shows ZFP expression in one or more brain region, wherein the brain region is selected from the group comprising, consisting, or consisting essentially of motor cortex, cortical regions, the entorhinal cortex, the hippocampus, the cerebellum, the globus pallidus, the thalamus, the midbrain, the caudate, the putamen, the substantia nigra, the pons, and the medulla.

[0228] Embodiment 69: The method of any one of embodiments 61-68 wherein the patient shows ZFP expression in a plurality of brain regions, wherein the brain region is selected from the group comprising, consisting, or consisting essentially of motor cortex, cortical regions, the entorhinal cortex, the hippocampus, the cerebellum, the globus pallidus, the thalamus, the midbrain, the caudate, the putamen, the substantia nigra, the pons, and the medulla.

[0229] Embodiment 70: The method of any one of embodiments 61-69 wherein the patient shows ZFP expression in thalamus, pons, cervical spinal cord, precentral gyrus, and temporal cortex.

[0230] Embodiment 71: The method of any one of embodiments 61-70 wherein the patient shows ZFP expression in more than one brain cell, the brain cell selected from the group comprising, consisting, or consisting essentially of a motor neuron, a sensory neuron, a dopaminergic neuron, a cholinergic neuron, a glutamatergic neuron, a GABAergic neuron, or a serotonergic neuron, a glial cell, optionally an oligodendrocyte, an astrocyte, a pericyte, a Schwann cell, or a microglial cell, an ependymal cell, a neuroepithelial cell, and combinations thereof.

[0231] Embodiment 72: The method of any one of embodiments 61-71 wherein the patient shows ZFP expression in a plurality of brain cells, wherein the brain cells are selected from the group comprising, consisting, or consisting essentially of a motor neuron, a sensory neuron, a dopaminergic neuron, a cholinergic neuron, a glutamatergic neuron, a GABAergic neuron, or a serotonergic neuron, a glial cell, optionally an oligodendrocyte, an astrocyte, a pericyte, a Schwann cell, or a microglial cell, an ependymal cell, a neuroepithelial cell, or combinations thereof.

[0232] Embodiment 73: The method of any one of embodiments 61-72 wherein in S100β-positive glial cells, ZFP expression is not detected.

[0233] Embodiment 74: The method of any one of embodiments 61-73 wherein ZFP expression is restricted to neurons by a hSYN1 promotor.

[0234] Embodiment 75: The method of any one of embodiments 61-74 wherein ZFP expression in S100β-positive glial cells is the same as prior to the introducing of the composition.

[0235] Embodiment 76: The method of any one of embodiments 61-75 wherein the patient shows PrP repression compared to prior to the introducing of the composition.

[0236] Embodiment 77: The method of any one of embodiments 61-76 wherein the patient shows PrP repression in the brain and/or spinal cord compared to prior to the introducing of the composition.

[0237] Embodiment 78: The method of any one of embodiments 61-77 wherein the patient shows PrP repression in ChAT-positive motor neurons in the spinal cord.

[0238] Embodiment 79: The method of any one of embodiments 61-78 wherein the patient shows PrP repression in more than one brain region, wherein the brain region is selected from the group comprising, consisting, or consisting essentially of motor cortex, cortical regions, the entorhinal cortex, the hippocampus, the cerebellum, the globus pallidus, the thalamus, the midbrain, the caudate, the putamen, the substantia nigra, the pons, the medulla and combinations thereof.

[0239] Embodiment 80: The method of any one of embodiments 61-79 wherein the patient shows PrP repression in a plurality of brain regions, wherein the brain region is selected from the group comprising, consisting, or consisting essentially of motor cortex, cortical regions, the entorhinal cortex, the hippocampus, the cerebellum, the globus pallidus, the thalamus, the midbrain, the caudate, the putamen, the substantia nigra, the pons, the medulla and combinations thereof.

[0240] Embodiment 81: The method of any one of embodiments 61-80 the patient shows PrP repression in a plurality of brain regions, wherein the brain region is selected from the group comprising, consisting, or consisting essentially of in thalamus, pons, cervical spinal cord, precentral gyrus, temporal cortex and combinations thereof.

[0241] Embodiment 82: The method of any one of embodiments 61-81 wherein the patient shows PrP repression in more than one brain cell, the brain cell selected from the group comprising, consisting, or consisting essentially of a motor neuron, a sensory neuron, a dopaminergic neuron, a cholinergic neuron, a glutamatergic neuron, a GABAergic neuron, or

a serotonergic neuron, a glial cell, optionally an oligodendrocyte, an astrocyte, a pericyte, a Schwann cell, or a microglial cell, an ependymal cell, a neuroepithelial cell, and combinations thereof.

[0242] Embodiment 83: The method of any one of embodiments 61-82 wherein the patient shows PrP repression in the brain and spinal cord.

[0243] Embodiment 84: The method of any one of embodiments 61-83 wherein the patient shows PrP repression in a plurality of NeuN-positive cells wherein ZFP expression is below the limit of detection of an In Situ Hybridization assay.

[0244] Embodiment 85: The method of any one of embodiments 61-84 wherein the subject is a human.

[0245] Embodiment 86: The method of any one of embodiments 61-85 wherein the patient shows PrP repression in a plurality of brain cells, wherein the brain cells are selected from the group comprising, consisting, or consisting essentially of a motor neuron, a sensory neuron, a dopaminergic neuron, a cholinergic neuron, a glutamatergic neuron, a GABAergic neuron, or a serotonergic neuron, a glial cell, optionally an oligodendrocyte, an astrocyte, a pericyte, a Schwann cell, or a microglial cell, an ependymal cell, a neuroepithelial cell, or combinations thereof.

[0246] Embodiment 87: A method of inhibiting expression of PrP in a human brain cell, comprising providing to a cell a composition of any one of claims 1-55 or a pharmaceutical composition according to claim 56, thereby inhibiting the expression of PrP in the cell.

[0247] Embodiment 88: The method of embodiment 87, wherein the human brain cell is a neuron, a glial cell, an ependymal cell, a neuroepithelial cell, an endothelial cell, or an oligodendrocyte.

[0248] Embodiment 89: The method of any one of embodiments 87-88, wherein the cell is in the brain of a patient suffering from or at risk of developing Alzheimer's disease, frontotemporal dementia, progressive supranuclear palsy, traumatic brain injury (TBI), seizure disorders, corticobasal degeneration (CBD), chronic traumatic encephalopathy (CTE), or another Tauopathy.

[0249] Embodiment 90: The method of any one of embodiments 87-89, comprising introducing into the cell the expression construct that expresses the fusion protein.

[0250] Embodiment 91: A method of treating a Tauopathy in a patient in need thereof, comprising administering to the patient a composition of any one of embodiments 1-55 or a pharmaceutical of embodiment 56.

[0251] Embodiment 92: The method of embodiment 91, wherein the composition is introduced to the patient via an intravenous, intrathecal, intracerebral, intracerebroventricular, intra-cisternal magna, intrahippocampal, intrathalamic, or intraparenchymal route.

[0252] Embodiment 93: The method of embodiment 91 or 92, wherein the Tauopathy is Alzheimer's disease, or frontotemporal dementia, progressive supranuclear palsy, traumatic brain injury (TBI), seizure disorders, corticobasal degeneration (CBD), or chronic traumatic encephalopathy (CTE).

[0253] Embodiment 94: The method of embodiment 91, 92 or 93, wherein the composition is introduced to the patient at a concentration of 1E14 vg/kg.

[0254] Embodiment 95: The method of any one of embodiments 91-94, wherein introducing into a subject in need thereof comprises administering the composition at a concentration of 1E14 vg/kg or between 1E12 vg/kg to 5E14 vg/kg.

[0255] Embodiment 96: The method of any one of embodiments 91-95, wherein introducing into a subject in need thereof comprises administering the composition by an intravenous, intrathecal, intracerebral, intracerebroventricular, intra-cisternal magna, intrahippocampal, intrathalamic, or intraparenchymal route.

[0256] Embodiment 97: The composition of any one of embodiments 1-55, the pharmaceutical composition of embodiment 107 or the host cell of embodiments 108-111 for use in a method of treating a Tauopathy in a patient in need thereof, comprising administering to the patient a composition of any one of claims 1-55.

[0257] Embodiment 98: The composition of any one of embodiments 1-55, the pharmaceutical composition of embodiment 107 or the host cell of embodiments 108-111 for use in a method of inhibiting expression of PrP in a human brain cell.

[0258] Embodiment 99: Use of composition of any one of embodiments 1-55, the pharmaceutical composition of embodiment 107 or the host cell of embodiments 108-111, for the manufacture of a medicament for inhibiting expression of PrP in a human brain cell and/or treating a Tauopathy in a patient.

[0259] Embodiment 100: Use of composition of any one of embodiments 1-55, the pharmaceutical composition of embodiment 107 or the host cell of embodiments 108-111 for the manufacture of a medicament for use in the method of any one of claims 91-96.

[0260] Non-limiting exemplary embodiments of the disclosure are further described below.

[0261] Embodiment 1: A composition comprising an AAV capsid polypeptide, comprising a peptide modification relative to the AAV9 capsid polypeptide set forth in SEQ ID NO: 1497, wherein: the peptide modification is in variable region 8 (VRVIII) and; the peptide modification comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of an amino acid sequence set forth in any one of SEQ ID NO: 263-1495, and the portion of the capsid polypeptide that is not the peptide modification comprises at least or about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 1497 and an expression construct encoding a fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the ZFP domain comprises DNA-binding recognition helix sequences as shown in a single row in Tables 1 and 3.

[0262] Embodiment 2: The composition of embodiment 1, wherein the peptide modification is in the region of the capsid polypeptide spanning positions 450 and 600, with numbering relative to SEQ ID NO: 1497.

[0263] Embodiment 3: The composition of embodiment 1, wherein the peptide modification is in the region of the capsid polypeptide spanning positions 587 and 590, with numbering relative to SEQ ID NO: 1497 or wherein the peptide modification is in the region of the capsid polypeptide spanning positions 384 and 386, with numbering relative to SEQ ID NO: 1497.

[0264] Embodiment 4: The composition of embodiment 1, 2 or 3, wherein the peptide modification comprises a 9-16 amino acid insertion to the AAV9 capsid polypeptide set forth in SEQ ID NO: 1497.

[0265] Embodiment 5: The composition of embodiment 1, 2, 3 or 4, wherein the peptide modification comprises an amino acid insertion of SEQ ID NO: 333, 417, 442, or 673.

[0266] Embodiment 6: The composition of embodiment 1, 2, 3, 4 or 5, wherein the ZFP domain comprises a DNA-binding recognition helix sequences as shown in a single row of Tables 1 and 3.

[0267] Embodiment 7: A composition comprising an AAV capsid polypeptide, comprising: a) a VP1 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1498; b) a VP2 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1499; c) a VP3 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1500, or d) a sequence having at least or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VP1, VP2 or VP3 proteins in a)-c), and

an expression construct encoding a fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the ZFP domain comprises DNA-binding recognition helix sequences as shown in a single row in Tables 1 and 3.

[0268] Embodiment 8: The composition of embodiment 7, wherein the fusion protein comprises a ZFR shown in Tables 2 and 4.

[0269] Embodiment 9: The composition of any one of embodiments 1-8, wherein the capsid crosses the blood brain barrier to a greater extent compared to an AAV vector comprising a capsid polypeptide comprising the sequence of amino acids set forth in SEQ ID NO: 1497.

[0270] Embodiment 10: The composition of any one of embodiments 1-9, wherein the capsid crosses the blood brain barrier by at least or about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400% or 500% greater extent compared to an AAV vector comprising a capsid polypeptide comprising the sequence of amino acids set forth in SEQ ID NO: 1497.

[0271] Embodiment 11: A composition of any one of embodiments 1-10 wherein the capsid encapsulates the fusion protein.

[0272] Embodiment 12: A composition comprising a vector comprising a nucleic acid molecule encoding SEQ ID NOS: 1498, 1499, or 1500 and an expression construct encoding a fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the ZFP domain comprises DNA-binding recognition helix sequences as shown in a single row in Tables 1 and 3.

[0273] Embodiment 13: A composition comprising a vector comprising a nucleic acid molecule encoding SEQ ID Nos: 1498, 1499, or 1500 and an expression construct encoding a fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the fusion protein comprises a ZFR shown in Tables 2 and 4.

[0274] Embodiment 14: A host cell, comprising the composition of any one of embodiments 1-13.

[0275] Embodiment 15: A method for introducing a heterologous coding sequence into a host cell, comprising contacting a host cell with the composition of any one of embodiments 1-13.

[0276] Embodiment 16: The method of embodiment 15, wherein the host cell is a human brain cell.

[0277] Embodiment 17: The method of embodiment 15 or 16, wherein contacting a host cell with the composition comprises administering the composition to a subject.

[0278] Embodiment 18: The method of embodiment 17, wherein administration of the composition to the subject effects treatment of a Tauopathy-associated disease or condition.

[0279] Embodiment 19: The method of any one of embodiments 15-19, wherein the method is in vitro or ex vivo.

[0280] Embodiment 20: Use of the composition of any one of claims 1-13 for the preparation of a medicament for treating a Tauopathy-associated disease or condition.

[0281] Non-limiting exemplary embodiments of the disclosure are further described below.

[0282] Embodiment 1: Use of a composition comprising an AAV capsid polypeptide, comprising a peptide modification relative to the AAV9 capsid polypeptide set forth in SEQ ID NO: 1497, wherein: the peptide modification is in variable region 8 (VRVIII) and; the peptide modification comprises a 9 amino acid insertion relative to the AAV9 capsid polypeptide set forth in SEQ ID NO: 1497, and comprises the sequence set forth in any one of SEQ ID Nos:263-1495; and the portion of the capsid polypeptide that is not the peptide modification comprises at least or about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 1497 and expression construct encoding a fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the ZFP domain comprises DNA-binding recognition helix sequences as shown in a single row in Tables 1 and 3 for inhibiting expression of PrP in a human brain cell and/or treating a Tauopathy in a patient.

[0283] Embodiment 2: Use of the composition of embodiment 1, wherein the peptide modification is in the region of the capsid polypeptide spanning positions 450 and 600, with numbering relative to SEQ ID NO: 1497.

[0284] Embodiment 3: Use of the composition of embodiment 1, wherein the peptide modification is in the region of the capsid polypeptide spanning positions 587 and 590, with numbering relative to SEQ ID NO: 1497 or wherein the peptide modification is in the region of the capsid polypeptide spanning positions 384 and 385, with numbering relative to SEQ ID NO: 1497.

[0285] Embodiment 4: Use of the composition of embodiment 1, 2 or 3, wherein the peptide modification comprises a 9-16 amino acid insertion to the AAV9 capsid polypeptide set forth in SEQ ID NO: 1497.

[0286] Embodiment 5: Use of the composition of embodiment 1, 2, 3 or 4, wherein the peptide modification comprises an amino acid insertion of SEQ ID NO: 333, 417, 442, or 673.

[0287] Embodiment 6: Use of the composition of embodiment 1, 2, 3, 4 or 5, wherein the fusion protein comprises a ZFR shown in Tables 2 and 4.

[0288] Embodiment 7: Use of a composition comprising an AAV capsid polypeptide, comprising: a) a VP1 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1498; b) a VP2 protein comprising the sequence of amino acids set forth in SEQ ID NO:1499; c) a VP3 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1500, or d) a sequence having at least or about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VP1, VP2 or VP3 proteins in a)-c), and an expression construct encoding a fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the ZFP domain comprises DNA-binding recognition helix sequences as shown in a single row in Tables 1 and 3 for inhibiting expression of PrP in a human brain cell and/or treating a Tauopathy in a patient.

[0289] Embodiment 8: Use of the composition of embodiment 7, wherein the fusion protein comprises a ZFR shown in Tables 2 and 4.

[0290] Embodiment 9: Use of the composition of any one of embodiments 1-8, wherein the capsid crosses the blood brain barrier to a greater extent compared to an AAV vector comprising a capsid polypeptide comprising the sequence of amino acids set forth in SEQ ID NO: 1497.

[0291] Embodiment 10: Use of the composition of any one of embodiments 1-9, wherein the capsid crosses the blood brain barrier by at least or about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400% or 500% greater extent compared to an AAV vector comprising a capsid polypeptide comprising the sequence of amino acids set forth in SEQ ID NO: 1497.

[0292] Embodiment 11: Use of the composition of any one of embodiments 1-10 wherein the capsid encapsulates the fusion protein.

[0293] Embodiment 12: Use of a composition comprising a vector comprising a nucleic acid encoding SEQ ID NOS: 1498, 1499, or 1500 and an expression construct encoding a fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the ZFP domain comprises DNA-binding recognition helix sequences as shown in a single row in Tables 1 and 3.

[0294] Embodiment 13: Use of a composition comprising a vector comprising a nucleic acid encoding SEQ ID NOS: 1498, 1499, or 1500 and an expression construct encoding a fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a human *PRNP* gene wherein the fusion protein comprising a ZFR shown in Tables 2 and 4.

[0295] Embodiment 14: Use of the composition of any one of embodiments 1-13, wherein the host cell is in one or more brain region comprising, consisting, or consisting essentially of motor cortex, cortical regions, the entorhinal cortex, the hippocampus, the cerebellum, the globus pallidus, the thalamus, the midbrain, the caudate, the putamen, the substantia nigra, the pons, the medulla and combinations thereof.

[0296] Embodiment 15: Use of the composition of any one of embodiments 1-14, wherein the host cell is one or more human brain cell selected from the group comprising, consisting, or consisting essentially of a motor neuron, a sensory neuron, a dopaminergic neuron, a cholinergic neuron, a glutamatergic neuron, a GABAergic neuron, or a serotonergic neuron, a glial cell, optionally an oligodendrocyte, an astrocyte, a pericyte, a Schwann cell, or a microglial cell, an ependymal cell, a neuroepithelial cell, or combinations thereof.

[0297] Embodiment 16: Use of the composition of any one of embodiments 1-15, wherein the composition is administered to a subject via an intravenous, intrathecal, intracerebral, intracerebroventricular, intra-cisternal magna, intrahippocampal, intrathalamic, or intraparenchymal route.

[0298] Embodiment 17: Use of the composition of any one of embodiments 1-16, wherein the composition is administered to a subject at a 1E14 vg/kg dose or a dose between 1E12 vg/kg to 5E14 vg/kg.

[0299] Embodiment 18: Use of the composition of any one of embodiments 1-17, wherein the method is in vitro or ex vivo.

EXAMPLES

Example 1: Screening of Anti-PRNP ZFP-Rs

[0300] In order to identify human specific ZFP-Rs that repress the expression of human *PRNP* gene, a library of ZFP-Rs were designed targeting human *PRNP* spanning from 500 bp upstream to 1350 bp downstream of the Transcription Start Site (TSS) and screened for *PRNP* repression activity. In this study, a KRAB domain sequence DAKSLTAWSR TLVTFKDVFV DFTREEWKLL DTAQQIVYRN VMLENYKNLV SLGYQLTKPD VILRLEKGEE PWLVEREIHQ ETHPDSETAF EIKSSV (SEQ ID NO: 262) was used as the transcription repressor and fused to the C-terminus of the ZFP domain.

[0301] The screening for human ZFP-Rs was performed in the SK-N-MC human neuroepithelial cell line. SK-N-MC cells express the *PRNP* gene at high levels and are thus appropriate for testing of ZFP-Rs that reduce *PRNP* expression. Each cell line was cultured in tissue culture flasks until confluency. The cells were plated on 96-well plates and were resuspended in Amaxa® SF solution. The cells were then mixed with ZFP-R mRNA 6 doses (3, 10, 30, 100, 300, and 1000 ng) and transferred to Amaxa® shuttle plate wells. The cells were transfected using the Amaxa® Nucleofector® device (Lonza; program CM-137). Eagle's MEM cell media was added to each well of the plate. The cells were transferred to a 96-well tissue culture plate and incubated at 37°C for 20 hours. The cells were then lysed and reverse transcription was performed using the C2CT kit following the manufacturer's instructions. TaqMan quantitative polymerase chain reaction (qPCR) was used to measure the expression levels of *PRNP*, which were normalized to the geometric mean of the expression levels of the housekeeping genes *ATP5B* and *EIF4A2*. A transfection with ZFP 65976 that is known not to target *PRNP* was used as a negative control. The maximum repression achieved was >99.8%, but ZFP-Rs were also identified that repressed *PRNP* to a lesser degree (*e.g.*, about 90%, about 75%, or about 50% at the highest dose or in some instances no repression). **FIG. 2** shows the screening data for exemplary human ZFP-Rs.

Example 2: AAV Production for Exemplary ZFP-Rs

[0302] Recombinant adeno-associated virus (rAAV) vectors were generated by the triple transfection method. Briefly, HEK293 cells were plated in ten-layer CellSTACK® chambers (Corning, Acton, MA) and grown for three days to a density of 80%. Three plasmids – (i) an AAV Helper plasmid containing the Rep and Cap genes, (ii) an Adenovirus Helper plasmid containing the adenovirus helper genes, and (iii) a transgene plasmid containing the sequence to be packaged flanked by AAV2 inverted terminal repeats were transfected into the cells using calcium phosphate. After three days, the cells were harvested. The cells were then

lysed by three rounds of freeze/thaw and the cell debris was removed by centrifugation. The rAAV was precipitated using polyethylene glycol. After resuspension, the virus was purified by ultracentrifugation overnight on a cesium chloride gradient. The virus was formulated by dialysis and then filter-sterilized. After adjusting the titer (virus genomes/ml) of all AAV batches by dilution with PBS + 0.001% Pluronic F-68, the AAVs were aliquoted to single use doses and stored at -80°C until use. After thawing, no refreezing was done.

Example 3: Human iPSC-Derived Neuron Culture and ZFP-R AAV Infection

[0303] Human iPSC-derived GABAergic neurons were purchased from Cellular Dynamics International and plated onto poly-L-ornithine- and laminin-coated 96-well plates at a density of 40,000 cells per well and maintained according to the manufacturer's instructions. The cells were infected with AAV expressing the desired ZFP-R at the indicated MOI 48 hours after plating and maintained for up to 31 days (50-75% media changes performed every 3-5 days). The cells were harvested at the end of the experimental period, RNA was isolated, and RT-qPCR was performed for gene expression analysis. **FIG. 3** shows *PRNP* expression levels in iPSC-derived neuronal culture following AAV ZFP-R delivery. For microarray analysis, the cells were transduced with 1E5 VGs/cell 48 hours after plating and harvested 19 days after viral transduction.

Example 4: Primary Mouse Neuron Culture and ZFP-R AAV Infection

[0304] Primary mouse cortical neurons (MCNs) were purchased from Gibco. Cells were plated onto poly-D-lysine (PDL)-coated 24-well plates at 300,000 cells/well, and maintained according to the manufacturer's specifications using Gibco Neurobasal Medium containing GlutaMAX™ I supplement, B27 supplement, and penicillin/streptomycin. 48 hours after plating (at DIV2), 30,000 cells/well in 24-well plates were infected with AAV ZFP-R at 3E5 VGs/cell to ensure a 100% transduction rate and harvested 7 days later (at DIV9; 50% media exchanges performed every 3-4 days) followed by RNA isolation and microarray analysis.

Example 5: Off-Target Evaluation of Anti-PRNP ZFP-Rs

[0305] To evaluate the potential off-target impact of the ZFP-Rs on global gene expression, microarray analysis (Clariom S Array) was performed on total RNA isolated from human iPSC-derived neurons and primary mouse cortical neurons treated with AAVs encoding representative ZFRs. Microarray analyses were performed following the manufacturer's protocol (Thermo Fisher Scientific), and the assay results were analyzed using TAC4 software.

[0306] **FIG. 3** shows microarray results of ZFP-Rs targeting human *PRNP* gene in human iPSC-derived neurons transduced with ZFP-Rs. **FIG. 3** shows the microarray results of

ZFP-Rs targeting human *PRNP* and their activity in primary mouse cortical neurons. Binding sites selected for human *PRNP*-targeting ZFP-R do not share homology with mouse *Prnp* gene locus, thus no prion-specific repression in mouse cortical neurons is expected.

Example 6: *In Vivo* Tolerability Study in Wildtype Mice

[0307] The tolerability of introducing AAV.PHP.B packaged ZFP-Rs targeting the human *PRNP* gene in wildtype C57BL/6 mouse was evaluated in the brain via RT-qPCR analysis on neuroinflammatory (*Gfap*, *Iba1)* and neuronal (*NeuN, Tubb3)* markers. Mice were euthanized 3 weeks after intravenously delivered with vehicle (formulation buffer) or test articles (AAV.PHP.B.ZFP-Rs). To harvest the tissue for subsequent analysis, mice were perfused with PBS, and each micro-dissected brain tissue was treated with RNALater for 24 hours, then the RNALater was removed, and tissue were flash-frozen in liquid nitrogen and maintained at -80°C until analysis. Reverse transcription was performed using the High-Capacity RT Kit (Thermo Fisher Scientific) kit following the manufacturer's instructions. TaqMan quantitative polymerase chain reaction (qPCR) was used to measure the expression levels of the ZFP-R, *Prnp, Gfap, Iba1, Rbfox3*, and *Tubb3*. Gene expression levels were normalized to the mean of the expression levels of the housekeeping genes *Atp5b* and *Eif4a2*, the scaled to the average value from the Vehicle -treated animals. **FIG. 4A** shows the RT-qPCR result from the brainstem. Most ZFP-Rs demonstrated minimal or no neuroinflammatory changes nor loss of neurons, except 89891, relative to the control group (vehicle treatment). A graph of body weight during the study shows ZFP-R 89891 treatment resulted in significant body weight loss, necessitating preterminal euthanasia (see **FIG. 4B)**. As expected, the human *PRNP*-targeting ZFP-Rs had no activity on reducing expression of mouse *PRNP* gene.

Example 7: *In Vivo* Pharmacology Study in Transgenic Mice

[0308] The pharmacological activity of AAV.PHP.B packaged ZFP-Rs targeting the human *PRNP* gene was tested in Tg25109 transgenic mice, which harbor the full length human *PRNP* transgene on the background of an endogenous mouse *Prnp* knock out. Mice were euthanized eight weeks after AAV administration. To harvest the tissue for subsequent analysis, mice were perfused with PBS, and each micro-dissected brain region was treated with RNALater for 24 hours, then the RNALater was removed, and tissue were flash-frozen in liquid nitrogen and maintained at -80°C until analysis. Reverse transcription was performed using the High-Capacity RT Kit (Thermo Fisher Scientific) kit following the manufacturer's instructions. TaqMan quantitative polymerase chain reaction (qPCR) was used to measure the expression levels of the ZFP-R, human *PRNP, Gfap, Iba1, Rbfox3*, and *Tubb3*. Gene expression levels (human *PRNP, Gfap, Iba1, Rbfox3*, and *Tubb3)* were normalized to the mean of the

expression levels of the housekeeping genes *Atp5b* and *Eif4a2* and scaled to the average value of the vehicle-treated animals. A clear dose-dependent (1e13 vs. 1e14 vg/kg) expression of ZFP-R (at the absolute level) and repression of *PRNP* mRNA expression was revealed by RT-qPCR analysis from various dissected brain regions at the bulk level (**FIG. 5**). At the high dose, all ZFP-Rs showed 40-80% *PRNP* repression depending on the brain regions.

[0309] Neuroinflammatory (*Gfap*, *Iba1)* and neuronal loss (*NeuN)* marker expression was also evaluated in the brain (**FIG. 6**). The results are consistent with the tolerability study using wildtype mice, with no ZFP-Rs showing severe neuroinflammatory signs nor neuron loss in the brain.

[0310] Brain hemispheres were removed (including the olfactory bulb and cerebellum) from all mice and placed in 10% NBF at RT for 22-24hr. After fixation, brain hemispheres were transferred to 0.01M PBS + 0.02% Sodium Azide, pH 7.4 ± 0.2 at 2-8°C. Brain hemispheres were then dissected into 6 coronal slabs (~2mm) and embedded into paraffin blocks and sectioned for multiplexed RNAscope-immunohistochemistry single cell analysis. A multiplexed approach combining *in situ* hybridization (ISH) to detect mRNA transcripts (*i.e.* ZFP-R and human *PRNP*) and immunohistochemistry (IHC) to label putative neurons (NeuN) was used. AAV-PHP.B-hSYN1-ZFP-Rs achieved >90% *PRNP* repression in neurons of thalamus at 1e14 vg/kg dose (**FIG. 7A** and **7B**).

[0311] Prion protein (PrP) expression was also evaluated from microdissected brain tissue and cerebrospinal fluid **(FIG. 8)**. Tissue from mice was flash frozen at necropsy. Tissues were lysed and homogenized with MP Biomedicals™ Lysing Matrix D 96-well tube assay. Total protein extracted from each brain tissue lysate was quantified by Bicinchoninic acid (BCA) assay Protein Assay Kit. Levels of PrP protein from brain tissues and CSF were quantified using a biotinylated anti-prion antibody and MSD GOLD 96-Well Small Spot Streptavidin Plate. PrP values were largely reduced (~80% in brainstem and ~75% in CSF) in ZFP-R treated animals at the 1e14 vg/kg dose when compared to the vehicle group.

Example 8: In Vivo Evaluation of Efficacy in Prion Disease Mouse Model with Mouse Surrogate ZFP-Rs that Target Mouse *Prnp* Gene

[0312] To evaluate the potential benefit of using a ZFP-R in an in vivo setting of prion disease, wildtype mice were intracerebrally injected with a misfolded strain (Rocky Mountain Laboratory; RML) of PrP^{Sc} prions. At either 60 or 122 days post inoculation (dpi), mice were then intravenously injected with a single 1e14 vg/kg dose of AAV.PHP.B encoding mouse *Prnp* ZFP-Rs or control treatments. Two ZFP-Rs were tested (ZFP-R 81193 or ZFP-R 81200, *see*, WO2021067864A1 which is hereby incorporated by reference in its entirety) that

repress the mouse *Prnp* gene in primary mouse cortical neurons >90% compared to control treatment. Control groups were injected either with vehicle or a 1e14 vg/kg dose of AAV.PHP.B encoding GFP. The ZFP-R and GFP transgenes were expressed from a neuron-specific Synapsin 1 promoter. One additional control group was included that was not inoculated with PrP^{Sc} or injected with a test article. Mice were monitored for body weight and general cageside behavior. Plasma neurofilament light chain (NfL) levels were assessed monthly. Any mouse that lost 20% body weight or showed predetermined signs of suffering was humanely euthanized. The data show that mice treated with ZFP-Rs had a significant extension in survival at both ZFP-R treatment timepoints compared to the control treatments. This correlated with increases and or stabilization of body weight, as well as a stabilization of plasma NfL levels for several months to over one year. **(FIG. 9).**

Example 9: Widespread Prion repression in the nonhuman primate brain following ZFP-R delivery using the engineered BBB penetrant capsid STAC-BBB

[0313] Adult cynomolgus macaques were injected with a single 2e13 vg/kg dose of an engineered AAV capsid that crosses the blood brain barrier in nonhuman primates. The AAV capsid delivered a ZFP-R targeting the primate *PRNP* gene. The ZFP-R was expressed from a ubiquitous CAG promoter. Animals were euthanized 19 days after test article administration. Brains were sectioned into 4mm coronal slices. For each animal, a total of 220 punch biopsies (2mm in diameter) were collected from across 35 brain regions (regions indicated on the x axis) for RNA analysis. Brain punches were incubated in RNALater for 24 hours at 4 degrees. The RNALater was removed and the punches were flash-frozen in liquid nitrogen and maintained at -80°C. Reverse transcription was performed using the High-Capacity RT Kit (Thermo Fisher Scientific) kit following the manufacturer's instructions. TaqMan quantitative polymerase chain reaction (qPCR) was used to measure the expression levels of the nonhuman primate *PRNP*. Gene expression levels were normalized to the mean of the expression levels of the housekeeping genes *ATP5B* and *EIF4A2*. The normalized *PRNP* value for each punch for a given brain region and location is scaled to the average value of the control-treated animals. The average of these scaled, normalized *PRNP* values for each region is shown for each animal. For each region, at least 2 punches were analyzed per animal. The data show that in the 35 brain regions assessed, *PRNP* levels were reduced compared to the control-treated animals. **(FIG. 10).**

CLAIMS

1. A fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a mammalian prion protein gene (*PRNP* gene).

2. The fusion protein of claim 1, wherein the target region is within about 1 kb or 500 bp of a transcription start site (TSS) in the *PRNP* gene.

3. The fusion protein of claim 1 or 2, wherein the *PRNP* gene is a human or non-human primate *PRNP* gene.

4. The fusion protein of any one of the preceding claims, wherein the ZFP domain comprises five or six zinc fingers and optionally represses expression of the *PRNP* gene by at least about 40%, 75%, 90%, 95%, or 99% with minimal to no detectable off-target binding or activity.

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

6. The fusion protein of any one of the preceding claims, wherein the ZFP domain is linked to the transcription repressor domain or the epigenetic silencing domain through a peptide linker.

7. The fusion protein of any one of the preceding claims, wherein the ZFP domain comprises a DNA-binding recognition helix sequence as shown in Tables 1 and 3.

8. The fusion protein of any one of the preceding claims, wherein the ZFP domain comprises the DNA-binding recognition helix sequences as shown in a single row in Tables 1 and 3.

9. The fusion protein of any one of the preceding claims, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequences linked as

shown in Tables 2 and 4.

10. The fusion protein of any one of the preceding claims, the fusion protein comprising, consisting, or consisting essentially of a sequence shown in Tables 2 and 4.

11. The fusion protein of any one of the preceding claims, wherein the ZFP domain binds to a Target Sequence shown in Tables 1 and 3.

12. A nucleic acid construct comprising a coding sequence for the fusion protein of any one of claims 1-11, wherein the coding sequence is linked operably to a transcription regulatory element.

13. The nucleic acid construct of claim 12, wherein the transcription regulatory element is a mammalian promoter that is constitutively active or inducible in a brain cell, and wherein the promoter is optionally a human synapsin I promoter.

14. A host cell comprising the nucleic acid construct of claim 12 or 13.

15. The host cell of claim 14, wherein the host cell is a human cell.

16. The host cell of claim 14, wherein the host cell is a brain cell or a pluripotent stem cell, wherein the stem cell is optionally an embryonic stem cell or an inducible pluripotent stem cell (iPSC).

17. A recombinant virus comprising the nucleic acid construct of claim 12 or 13.

18. The recombinant virus of claim 17, wherein the recombinant virus is recombinant adeno-associated virus (AAV), optionally of serotype AAV1, AAV2, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV11, AAVrh10, AAVrh39, AAVrh74, or STAC-BBB, and natural or engineered derivatives thereof.

19. A pharmaceutical composition comprising the nucleic acid construct of claim 12 or 13, or the recombinant virus of claim 17 or 18, and a pharmaceutically acceptable carrier.

20. A method of inhibiting expression of prion protein (PrP) in a mammalian brain cell, comprising introducing into the cell a fusion protein of any one of claims 1-11, optionally through introduction of a nucleic acid construct of any one of claim 12 or 13 or the recombinant virus of claim 14 or 15, thereby inhibiting the expression of PrP in the cell.

21. The method of claim 20, wherein the mammalian brain cell is a human, non-human primate, rodent, or murine cell.

22. The method of claim 20 or 21, wherein the mammalian brain cell is a neuron, a glial cell, an ependymal cell, or a neuroepithelial cell.

23. The method of any one of claims 20-22, wherein the cell is in the brain of a patient suffering from or at risk of developing prion disease, wherein the prion disease is optionally familial, sporadic, or acquired prion disease.

24. The method of claim 23, wherein the prion disease is Creutzfeldt-Jakob Disease (CJD), sporadic CJD, variant CJD, Gerstmann-Straussler-Scheinker Syndrome (GSS), Fatal Familial Insomnia (FFI), sporadic Fatal Insomnia (sFI), Kuru, or variably protease-sensitive prionopathy (VPSPr).

25. The method of any one of claims 20-24, comprising introducing into the cell the recombinant virus of claim 17 or 18.

26. A method of treating or preventing a neurodegenerative disease in a patient, comprising administering to the patient a recombinant AAV of claim 18.

27. The method of claim 26, wherein the neurodegenerative disease is prion disease, optionally wherein the prion disease is familial, sporadic, or acquired prion disease.

28. The method of claim 26 or 27, wherein the AAV is introduced to the patient via intravenous, intrathecal, intracerebroventrical, intra-cisternal magna, or intrathalamic injection, or injection into any cerebral region.

29. The method of claim 27 or 28, wherein the prion disease is Creutzfeldt-Jakob Disease (CJD), sporadic CJD, variant CJD, Gerstmann-Straussler-Scheinker Syndrome (GSS), Fatal Familial Insomnia (FFI), sporadic Fatal Insomnia (sFI), Kuru, or variably protease-sensitive prionopathy (VPSPr).

30. A fusion protein of any one of claims 1-11, a nucleic acid construct of claim 12 or 13, or a recombinant virus of claim 17 or 18 for use in the method of any one of claims 20-29.

31. Use of a fusion protein of any one of claims 1-11, a nucleic acid construct of claim 9 or 10, or a recombinant virus of claim 17 or 18 for the manufacture of a medicament for treating a patient in the method of any one of claims 20-29.

32. A composition comprising: 1) an adeno-associated virus (AAV) capsid protein comprising an amino acid sequence, wherein the amino acid sequence comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the amino acid sequence set forth in any one of SEQ ID NOS: 333, 417, 442, or 673; and 2) an expression construct comprising a coding sequence for a fusion protein, wherein the fusion protein comprises a zinc finger protein (ZFP) domain and a transcription repressor domain or epigenetic silencing domain, wherein the ZFP domain binds to a target region of a mammalian prion protein gene (*PRNP* gene).

33. The composition of claim 32, wherein the AAV capsid protein encapsulates the expression construct.

34. The composition of any one of the preceding claims 32-33, wherein the amino acid sequence comprises at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of the amino acid sequence set forth in SEQ ID NO: 333 inserted into SEQ ID. NO: 1497, optionally wherein the insertion is between amino acids 587 and 590.

35. The composition of any one of the preceding claims 32-34, the AAV capsid protein comprising one or more amino acid sequences selected from the group comprising, consisting, or consisting essentially of SEQ ID NO: 1498, 1499, or 1500.

36. The composition of any one of the preceding claims 32-35, the AAV capsid protein comprising a VP1 protein comprising a sequence of amino acids set forth in SEQ ID NO:

1498; a VP2 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1499; and a VP3 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1500.

37. The composition of any one of the preceding claims 32-36, the AAV capsid protein comprising the amino acid sequence having at least 80%, 85%, 90%, 95%, 99% or 100% identity to SEQ ID NO: 1498.

38. The composition of any one of the preceding claims 32-37, the AAV capsid protein comprising the amino acid sequence having at least 80%, 85%, 90%, 95%, 99% or 100% identity to SEQ ID NO: 1499 or to SEQ ID NO: 1500.

39. The composition of any one of the preceding claims 32-38, wherein the target region is within about 1 kb or 500 bp of a transcription start site (TSS) in the *PRNP* gene.

40. The composition of any one of the preceding claims 32-39, wherein the fusion protein represses expression of the *PRNP* gene by at least about 40%, 75%, 90%, 95%, or 99% with no or minimal detectable off-target binding or activity.

41. The composition of any one of the preceding claims 32-40, the transcription repressor domain comprising a KRAB domain, wherein the KRAB domain optionally is from a human KOX1 protein.

42. The composition of any one of the preceding claims 32-41, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequence as shown in Tables 1 and 3.

43. The composition of any one of the preceding claims 32-41, the ZFP domain comprising, consisting, or consisting essentially of DNA-binding recognition helix sequences shown in a single row in Tables 1 and 3.

44. The composition of any one of the preceding claims 32-43, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequences linked as shown in Tables 2 and 4.

45. The composition of any one of the preceding claims 32-44, the ZFP domain binds to a Target Sequence shown in Tables 1 and 3.

46. The composition of any one of the preceding claims 32-45, the fusion protein comprising, consisting, or consisting essentially of a sequence shown in Tables 2 and 4.