DELIVERY OF TAU REPRESSORS USING THE BLOOD-BRAIN BARRIER PENETRANT CAPSID

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application is related to U.S. Provisional Application 63/564,798, filed on March 13, 2024, U.S. Provisional Application 63/640,806, filed on April 30, 2024, U.S. Provisional Application 63/643,329, filed on May 6, 2024 and U.S. Provisional Application 63/719,334, filed on November 12, 2024. The contents of all 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 1525 sequences. The attached sequence listing is 1.55 MB in size, has the file name "9135511616.xml", and has a production date of March 12, 2025.

BACKGROUND OF THE INVENTION

[0003] Microtubule-associated protein tau (MAPT), also known as tau, plays an important role in certain brain pathologies. The aggregation of misfolded tau into neurofibrillary tangles (NFTs) and other pathological tau inclusions is implicated in a number of neurodegenerative conditions collectively referred to as tauopathies. These include Alzheimer's disease (AD), frontotemporal dementia (FTD), progressive supranuclear palsy (PSP), intractable genetic epilepsies (*e.g.,* Dravet syndrome), traumatic brain injury (TBI), corticobasal degeneration (CBD), and chronic traumatic encephalopathy (CTE). *See, e.g.,* Benussi et al., *Front Aging Neurosci.* (2015) 7:171; Gheyara et al., *Ann Neurol.* (2014) 76:443-56; Scholz and Bras, *Int J Mol Sci.* (2015) 16(10):24629-55; and McKee et al., *Brain Pathol.* (2015) 25(3):350-64.

[0004] It has been suggested that tau has prion-like properties. Several studies show that hyperphosphorylation of tau can cause tau misfolding. Misfolded tau aggregates can spread throughout the brain (Takeda et al., *Nat Comm.* (2015) 6:8490; Hyman, *Neuron* (2014) 82:1189; de Calignon et al., *Neuron* (2012) 73:685-97). These aggregates may be the initial step in the formation of NFTs found in tauopathies. Although NFTs are restricted to the entorhinal cortex and medial temporal lobe in the early stages of AD, by the time severe clinical symptoms appear, NFTs are widespread

throughout the brain. Both NFTs and amyloid-beta plaques are found in patients with AD, and amyloid deposition has been shown to increase tau pathology and deposition in distal brain regions (Bennett et al., *Am J Pathol.* (2017) 187(7):1601-12). Regional tau accumulation and spreading is closely linked to neuronal loss in rodent models and human disease (Pooler et al., *Acta Neuropathol Commun.* (2015) 3:14; La Joie et al., *Sci Transl Med.* (2020) 12:524). In addition to the neurotoxicity exerted by the accumulation of aggregated tau, soluble oligomeric forms of tau appear to be toxic as well (Guerrero-Munoz et al., *Front Cell Neurosci.* (2015) 9:464). Soluble misfolded endogenous tau seems to play a role as a mediator of neurotoxicity in various neuronal stress conditions.

[0005] Reduction of endogenous tau has been shown to be beneficial for AD-like pathology in different genetic mouse models (Roberson et al., *Science* (2007) 316:750-4; DeVos et al., *Sci Transl Med.* (2017) 9(374):eaag0481; Wegmann et al., *EMBO J.* (2015) 1-14). Decreasing tau levels in the brain also appears to protect against stress-induced and seizure-induced neuronal damage, and against learning and memory deficits resulting from traumatic brain injury (Lopes et al., *PNAS* (2016) 113:e3755-63; Gheyara, *supra*; DeVos et al., *J Neurosci.* (2013) 33(31):12887-97; Cheng et al., *PLoS One* (2014) 9(12):e115765). However, there has been no effective treatment for tauopathies. Tau knock-down *in vivo* has been achieved through administration of antisense oligonucleotides (ASOs) that bind tau mRNA and prevent its translation (DeVos (2017) *ibid*; DeVos et al., *Neurotherapeutics* (2013) 10(3):486-97) or by intravenous injections of anti-tau antibodies (Asuni et al., *J Neurosci.* (2007) 27:9115-29; Ittner et al., *J Neurochem.* (2015) 132:135-45; Herrmann et al., *J Neurochem.* (2015) 132:1-4; Yanamandra et al., *Neuron* (2013) 80(2):402-14). Although both approaches may facilitate tau protein reduction in the brain, they require chronic administrations for the lifetime of the patient. Antibodies have poor blood-brain barrier and cell membrane permeability, which can limit both their spread within the central nervous system and their ability to engage intraneuronal tau. Moreover, the development of anti-tau antibody therapeutics has been difficult because the identity and number of pathogenic tau species is currently unknown and may vary among tauopathies.

[0006] A third approach for reducing endogenous tau involves repressing *MAPT* using a zinc finger protein (ZFP) linked to a transcription repressor domain (*i.e.*, ZFR). *MAPT*-targeting ZFRs have shown promise for the treatment of tau-related human brain diseases from experiments in a mouse model of tauopathy (Wegmann et al., *Sci Adv.* (2021) 7(12):eabe1611) or human models (See International Patent Publication

WO 21/151012 which is herein incorporated by reference in its entirety). In mouse and rodent models, there has been evidence showing mild immune responses to adeno-associated virus (AAV). In preclinical studies, it was originally believed that these responses were too weak and transient to negatively impact sustained transduction. However, translation of rodent models to human treatments has not always been successful. As such, there remains a need for immunomodulatory therapies to overcome or bypass immune responses and for developing strategic approaches toward engineering stealth AAV vectors that can circumvent immunity.

[0007] International Patent Publication WO 21/151012 (which is herein incorporated by reference in its entirety) shows tau-repressing activity of 48 ZFP-TFs selected from a library of 370 ZFP-TFs directed to human MAPT transcripts. 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.

[0008] 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.

[0009] Given the significant role of tau in brain pathologies and the lack of effective treatment, there is an urgent need to develop therapeutic compositions targeting this protein for the prevention and treatment of tauopathies, including AD.

SUMMARY OF THE INVENTION

[0010] Generally, the problem to be solved is how to deliver compositions comprising recombinant adeno-associated virus (rAAV) particles and a ZFP capable of repressing *MAPT* expression to the central nervous system of a mammal (*e.g.,* human).

[0011] Disclosed are compositions 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 SEQ ID NO: 71; 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, wherein the ZFP domain binds to a target region of a human microtubule-associated protein tau (*MAPT*) gene. In some embodiments, the AAV capsid protein encapsulates the expression construct. In some embodiments, 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: 71 inserted into SEQ ID. NO: 1234.

[0012] 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 NOS: 1235, 1236, 1237. In some embodiments, the AAV capsid protein comprising a VP1 protein comprising a sequence of amino acids set forth in SEQ ID NO: 1235; a VP2 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1236; and a VP3 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1237. 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: 1235. 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: 1236 or 1237. In some embodiments, the target region within 1.5 kb of a transcription start site (TSS) in the *MAPT* gene. In some embodiments, the target region within 1000 bps upstream of the TSS, and/or within 500 bps downstream of the TSS of the *MAPT* gene. In some embodiments, the fusion protein represses expression of the *MAPT* 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 shown in a single row in FIGs 2 or 4. In some embodiments, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequences linked as shown in FIG. 3, or FIG. 5. In some embodiments, the ZFP domain comprising, consisting, or consisting essentially of an amino acid sequence selected from SEQ ID NOs: 1322-1429, 1430-1481 and 1483-1524. In some embodiments, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4 (*i.e.*, ZFP ID 73133). In some embodiments, the ZFP domain binds to a target sequence shown in FIG. 2 or FIG. 4. The composition of any one of claims 1-16, the ZFP domain binds to a target sequence shown in SEQ ID NO: 1311. In some embodiments, the fusion protein comprising, consisting, or consisting essentially of a sequence shown in FIG. 3 or FIG. 5. In some embodiments, the fusion protein comprising a sequence having at least 80%, 85%, 90% or 99% identity to SEQ ID NO: 1509. In some embodiments, the fusion protein under control of the human Synapsin1 (hSYN1) promoter. In some embodiments, the fusion protein comprising SEQ ID NO: 1509 (*i.e.*, ZFP ID 73133). In some embodiments, wherein the fusion protein is expressed in brain cells or spinal cord cells of a patient that has been injected with the composition.

[0013] In some embodiments, the fusion protein is expressed in one or in more than one brain cell of the patient wherein the brain cell is 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, the fusion protein is expressed in one or in more than one brain region of the patient 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 or combinations thereof. In some embodiments, the fusion protein is expressed in one or in more than one brain region of the patient wherein the brain region is selected from the group comprising, consisting, or consisting essentially of the brainstem, spinal cord, motor cortex, temporal cortex, thalamus or combinations thereof. In some embodiments, the fusion protein is expressed in the pons region of the

brainstem, cervical level of the spinal cord, precentral gyrus region of the motor cortex, temporal cortex, and thalamus. In some embodiments, the fusion protein is not expressed in S100β-positive glial cells. In some embodiments, the fusion protein is expressed in ChAT-positive motor neurons in the spinal cord. In some embodiments, the fusion protein is administered to a patient via a one-time intravenous, intrathecal, intracerebral, intracerebroventricular, intra-cisternal magna, intrahippocampal, intrathalamic, or intraparenchymal administration. In some embodiments, the fusion protein is administered at a dose between 1E12 vg/kg to 5E14 vg/kg or therapeutically effective amount. In some embodiments, the fusion protein is administered at a 1E14 vg/kg dose or therapeutically effective amount.

[0014] In another aspect, disclosed are pharmaceutical compositions 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 SEQ ID NO: 71; 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, wherein the ZFP domain binds to a target region of a human microtubule-associated protein tau (*MAPT*) gene. In some embodiments, the pharmaceutical composition comprises an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509,

[0015] In another aspect, disclosed are methods of inhibiting expression of tau in a human brain cell, comprising providing to a cell the composition described above (including but not limited to, an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509) or the pharmaceutical composition described above, thereby inhibiting the expression of tau in the cell. Disclosed is a method of inhibiting expression of tau in a human brain cell, comprising introducing into a patient in need thereof the composition described above (including but not limited to, an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509) or the pharmaceutical composition described above, thereby inhibiting the expression of tau in the patient. In some methods, the human brain cell is selected from the group comprising, consisting, or consisting essentially of a neuron, a glial cell, an ependymal cell, a neuroepithelial cell, an endothelial cell, or an oligodendrocyte. In some methods,

the human brain 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.

[0016] Disclosed is a method of treating a tauopathy in a patient in need thereof, comprising introducing into a patient in need thereof the composition described above (including but not limited to, an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509) or the pharmaceutical composition described above.

[0017] In some methods, the composition is introduced to the patient via an intravenous, intrathecal, intracerebral, intracerebroventricular, intra-cisternal magna, intrahippocampal, intrathalamic, or intraparenchymal route. In some methods, the composition is introduced to the patient at a dose of between 1E12 vg/kg to 5E14 vg/kg or therapeutically effective amount. In some methods, the composition is introduced to the patient at a dose of 1E14 vg/kg or therapeutically effective amount. In some methods, the AAV capsid penetrates across the patient blood brain barrier. In some methods, the patient has fusion protein expression. In some methods, the patient has fusion protein expression in brain cells and/or spinal cord cells. In some methods, the patient has fusion protein expression in one or in more than one brain cell wherein the brain cell is 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 methods, the patient has fusion protein expression in one or in more than one brain region of the patient 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 or combinations thereof. In some methods, the patient has fusion protein expression in one or in more than one brain region of the patient wherein the brain region is selected from the group comprising, consisting, or consisting essentially of the brainstem, spinal cord, motor cortex, temporal cortex, thalamus or combinations thereof. In some methods, the patient has fusion protein expression in the pons region of the brainstem, cervical level of the spinal cord, precentral gyrus region

of the motor cortex, temporal cortex, and thalamus. In some methods, the fusion protein is not expressed in S100β-positive glial cells. In some methods, the fusion protein is expressed in ChAT-positive motor neurons in the spinal cord. In some methods, the fusion protein expression is restricted to neurons by a hSYN1 promotor.

[0018] In some methods, the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition. In some methods, the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition in more than one brain cell of the subject wherein the brain cell is 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 methods, the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition in more than one brain region of the patient 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 or combinations thereof. In some methods, the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition in more than one brain region of the patient wherein the brain region is selected from the group comprising, consisting, or consisting essentially of the brainstem, spinal cord, motor cortex, temporal cortex, thalamus or combinations thereof. In some methods, the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition in the pons region of the brainstem, cervical level of the spinal cord, precentral gyrus region of the motor cortex, temporal cortex, and thalamus. In some methods, the patient does not show tau repression compared to prior to introducing of the composition or pharmaceutical composition in S100β-positive glial cells. In some methods, the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition in ChAT-positive motor neurons in the spinal cord. In some methods, the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition in a plurality of NeuN-positive cells wherein fusion protein expression is below the limit of detection of an In Situ Hybridization assay. In some methods, the patient is a human.

[0019] Disclosed is use of a composition described above (including but not limited to, an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509) for the manufacture of a medicament for use in a method described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] **FIG. 1(A)** is a schematic showing the packaging of a transgene encoding a Zinc Finger Repressor (ZFR) targeting the *MAPT* gene into an AAV capsid protein STAC-BBB (herein defined as SEQ ID NO: 1235). The packaged ZFR in the AAV capsid protein can be administered to a subject for the treatment of a tauopathy.

[0021] **FIG. 1(B)** is a schematic showing the intravenous administration of an AAV capsid protein harboring the *MAPT*-targeted ZFR transgene to a human subject (left panel). The AAV capsid protein crosses the blood-brain barrier and transduces cells in the brain, delivering the *MAPT*-targeted ZFR transgene to the nuclei of cells in the brain (right panel). Shown is an example neuron in the brain transduced with STAC-BBB and stably expressing the ZFR protein. The ZFR binds to its target DNA sequence in the *MAPT* locus and represses transcription of the *MAPT* gene, reducing the *MAPT* mRNA and thereby tau protein.

[0022] **FIG. 1(C)** shows how a *MAPT*-targeted ZFR delivered by an AAV capsid protein to a neuron leads to the reduction of tau protein inside neurons. The lower intracellular level of tau enables clearance and turnover of any toxic, pathological and mislocalized tau protein species that are associated with neurotoxicity, neurodegeneration, and propagation of misfolded tau in the brain of tauopathy patients. The reduction and clearance of these toxic tau species enables restoration of neuron health and function.

[0023] **FIG. 2** is a table showing exemplary ZFP. 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 four, five, or six finger ZFP shown (*i.e.*, F1-F4, F1-F5, or F1-F6). This figure also indicates illustrative peptide linker sequences as shown in **Table 1** 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). SEQ ID NO for each sequence is shown in parenthesis.

[0024] **FIG. 3** is a table showing illustrative full protein sequences for ZFRs comprising the ZFPs shown in **FIG. 2**. DNA-binding recognition helix sequences are in

boldface. Zinc finger linkers are underlined, whereas interdomain linkers are double underlined. SEQ ID NO for each sequence is shown in parenthesis. The capsid carries an expression construct that encodes a sequence with a leading M as showing in SEQ ID NOS: 1430-1481. But the ZFR that is delivered, *i.e.*, the functional protein, may have the leading M in SEQ ID NOS: 1430-1481 removed.

[0025] **FIG. 4** is a table showing exemplary R→Q ZFPs. 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 four, five, or six finger ZFP shown (*i.e.,* F1-F4, F1-F5, or F1-F6). This figure also indicates illustrative peptide linker sequences as shown in Table 1 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). SEQ ID NO for each sequence is shown in parenthesis.

[0026] **FIG. 5** is a table showing illustrative full protein sequences for R→Q ZFRs comprising the ZFPs shown in FIG. 4. DNA-binding recognition helix sequences are in boldface. Zinc finger linkers are underlined, whereas interdomain linkers are double underlined. SEQ ID NO for each sequence is shown in parenthesis. The capsid carries an expression construct that encodes a sequence with a leading M as showing in SEQ ID NOS: 1483-1524. But the ZFR that is delivered, *i.e.*, the functional protein, may have the leading M in SEQ ID NOS: 1483-1524 removed.

[0027] **FIG. 6(A)** is a schematic showing the study design evaluating STAC-BBB with a MAPT-targeted ZFR in adult cynomolgus macaques. Animals received a single intravenous administration of STAC-BBB (SEQ ID NO: 1235) harboring a MAPT-targeted ZFR (SEQ ID NO: 1509 under the control of the human Synapsin1 (hSYN1) promoter at one of three doses: 5E12 vg/kg, 2E13 vg/kg, or 1E14 vg/kg. The in-life portion of the study was 28 days. At necropsy, brains were hemisected and coronal sections were collected for subsequent analysis by either multiplexed in situ hybridization (ISH) with immunohistochemistry (IHC) or RT-qPCR for bulk gene expression.

[0028] **FIG. 6(B)** is a heat-mapped diagram depicting expression values of the MAPT-targeted ZFR in various punch biopsies taken from the right brain hemisphere of animals treated with a vehicle control or one of three doses of STAC-BBB encapsulating an expression construct encoding a MAPT- targeted ZFR (SEQ ID NO:

1509) (hereinafter, STAC-BBB/MAPT-ZFR (SEQ ID NO: 1509)). The coronal brain level is shown at the top, ranging from the most rostral (Level 4) to the most caudal (Level 10). A representative animal from each dose group is shown for each level, with the top row showing data from a vehicle-treated animal, the second row showing the data for a 5E12 vg/kg treated animal, the third row showing the data for a 2E13 vg/kg treated animal, and the last row showing the data for a 1E14 vg/kg treated animal. The expression level was measured by RT-qPCR and is reported in ZFR transcripts per ng RNA, ranging from 1 to >1000 transcripts/ngRNA. The results show dose-dependent and widespread expression of STAC-BBB delivered MAPT-targeted ZFR throughout all levels of the brain and most brain regions analyzed. Vehicle-treated animals had undetectable levels of ZFR.

[0029] **FIG. 6(C)** shows exemplary ZFR and MAPT RT-qPCR expression results from two brain regions: thalamus (top) and pons (bottom). The left panels show ZFR expression in units of ZFR transcripts per ng of RNA. The right panels show tau mRNA (MAPT) expression normalized to the geometric mean of two housekeeping genes and scaled to the mean of the vehicle-treated group. The bar graphs represent the average expression value for the three animals that were treated at each dose level. Shown from left to right in each panel are the vehicle group; and 5E12 vg/kg, 2E13 vg/kg, and 1E14 vg/kg of STAC-BBB/MAPT-ZFR (SEQ ID NO: 1509). The results show dose-dependent increase in ZFR expression that correlates with a dose-dependent decrease in MAPT mRNA.

[0030] **FIG. 6(D)** is an exemplary image of the pons region of the brainstem showing in situ hybridization (ISH) and immunohistochemistry (IHC) staining results for ZFR transcripts, MAPT transcripts, and NeuN protein. The top panels show the results from a vehicle-treated animal. The bottom panels show the results from an animal treated with STAC-BBB/MAPT-ZFR (SEQ ID NO: 1509) at the 1E14 vg/kg dose. The left panels show the signal for an ISH probe that detects MAPT transcripts (white) overlaid with the IHC signal from an anti-NeuN antibody stain (purple). The middle panels show a magnified region (indicated by a small white outlined box in the left panels) with the signal obtained using an ISH probe that detects ZFR transcripts (green). The right panels show the same magnified region as in the middle panels for the ISH probe that detects MAPT transcripts (white) overlaid with the IHC signal from an anti-NeuN antibody stain (purple). The results show that the pons region of the brainstem of the STAC-BBB encapsulating an expression construct encoding a MAPT ZFR showed

high levels of ZFR expression and reduced MAPT expression in NeuN-positive neurons. At the single-cell level, ZFR transcript expression was strongly correlated with substantially reduced MAPT transcript levels in individual neurons

[0031] **FIG. 6(E)** is an exemplary image from the cervical level of the spinal cord showing in situ hybridization (ISH) and immunohistochemistry (IHC) staining results for ZFR transcripts, MAPT transcripts, NeuN protein, and ChAT protein. The top panels show the results from a vehicle-treated animal. The bottom panels show the results from an animal treated with STAC-BBB/MAPT-ZFR (SEQ ID NO: 1509) at the 1E14 vg/kg dose. The left-most panels show the signal for an ISH probe that detects MAPT transcripts (white) overlaid with the IHC signal from an anti-ChAT antibody stain (orange). The second-from-left-most panels show the signal for an ISH probe that detects ZFR transcripts (green) overlaid with the IHC signal from an anti-NeuN antibody stain (purple). The middle panels show a magnified region (indicated by a small white outlined box in the left panels) with the signal obtained using an ISH probe that detects ZFR transcripts (green). The second- from-right-most panels show the same magnified region as in the middle panels for the IHC signal from an anti-ChAT antibody stain (orange). The right-most panels show the same magnified region as in the middle panels for the ISH probe that detects MAPT transcripts (white) overlaid with the NeuN signal from an anti-NeuN antibody stain (purple). The results show that the cervical level of the spinal cord from an NHP treated with STAC-BBB/MAPT-ZFR (SEQ ID NO: 1509) showed high levels of ZFR expression and reduced MAPT expression in NeuN-positive, ChAT-positive motor neurons. At the single-cell level, ZFR transcript expression was strongly correlated with substantially reduced MAPT transcript levels in individual ChAT-positive motor neurons.

[0032] **FIG. 6(F)** is an exemplary image from the precentral gyrus region of the motor cortex showing in situ hybridization (ISH) and immunohistochemistry (IHC) staining results for ZFR transcripts, MAPT transcripts, NeuN protein, and S100β protein. The top panels show the results from a vehicle-treated animal. The bottom panels show the results from an animal treated with STAC-BBB/MAPT-ZFR (SEQ ID NO: 1509) at the 1E14 vg/kg dose. The left panels show the signal for an ISH probe that detects MAPT transcripts (white) overlaid with the IHC signal from an anti-NeuN antibody stain (purple). The middle panels show a magnified region (indicated by a small white outlined box in the left panels) with the signal obtained using an ISH probe that detects MAPT transcripts (white) overlaid with the IHC signals from an anti-NeuN

antibody stain (purple) and from an anti-S100β antibody stain (orange). The right panels show the same magnified region as in the middle panels for the ISH probe that detects ZFR transcripts (green) and MAPT transcripts (white) overlaid with the IHC signal from an anti-NeuN antibody stain (purple) and from an anti-S100β antibody stain (orange). The results show that the precentral gyrus of the motor cortex from an NHP treated with STAC-BBB encapsulating an expression construct encoding a MAPT-ZFR displayed high levels of ZFR expression and reduced MAPT expression in NeuN-positive neurons. Glial cells (labeled by the S100β stain) in the STAC-BBB encapsulating an expression construct encoding a MAPT-ZFR treated animal retained MAPT transcripts due to ZFR expression being restricted to neurons by the hSYN1 promoter. At the single-cell level, ZFR transcript expression was strongly correlated with substantially reduced MAPT transcript levels in individual NeuN-positive neurons in the precentral gyrus of the motor cortex.

[0033] **FIG. 6(G)** is an exemplary image from the temporal cortex showing in situ hybridization (ISH) and immunohistochemistry (IHC) staining results for ZFR transcripts, MAPT transcripts, NeuN protein, and S100β protein. The top panels show the results from a vehicle-treated animal. The bottom panels show the results from an animal treated with STAC-BBB/MAPT-ZFR (SEQ ID NO: 1509) at the 1E14 vg/kg dose. The left panels show the signal for an ISH probe that detects MAPT transcripts (white) overlaid with the IHC signal from an anti-NeuN antibody stain (purple). The middle panels show a magnified region (indicated by a small white outlined box in the left panels) with the signal obtained using an ISH probe that detects MAPT transcripts (white) overlaid with the IHC signals from an anti-NeuN antibody stain (purple) and from an anti-S100β antibody stain (orange). The right panels show the same magnified region as in the middle panels for the ISH probe that detects ZFR transcripts (green) and MAPT transcripts (white) overlaid with the IHC signal from an anti-NeuN antibody stain (purple) and from an anti-S100β antibody stain (orange). The results show that the temporal cortex from an NHP treated with STAC-BBB/MAPT-ZFR (SEQ ID NO: 1509) displayed high levels of ZFR expression and reduced MAPT expression in NeuN-positive neurons. Glial cells (labeled by the S100β stain) in the STAC-BBB/MAPT-ZFR (SEQ ID NO: 1509) treated animal retained MAPT transcripts due to ZFR expression being restricted to neurons by the hSYN1 promoter. At the single-cell level, ZFR transcript expression was strongly correlated with substantially reduced MAPT transcript levels in individual NeuN-positive neurons in the temporal cortex.

[0034] **FIG. 6(H)** is an exemplary image from the thalamus showing in situ hybridization (ISH) and immunohistochemistry (IHC) staining results for ZFR transcripts, MAPT transcripts, NeuN protein, and S100β protein. The top panels show the results from a vehicle-treated animal. The bottom panels show the results from an animal treated with STAC-BBB/MAPT-ZFR (SEQ ID NO: 1509) at the 1E14 vg/kg dose. The left panels show the signal for an ISH probe that detects MAPT transcripts (white) overlaid with the IHC signal from an anti-NeuN antibody stain (purple). The middle panels show a magnified region (indicated by a small white outlined box in the left panels) with the signal obtained using an ISH probe that detects MAPT transcripts (white) overlaid with the IHC signal from an anti-NeuN antibody stain (purple) and from an anti-S100β antibody stain (orange). The right panels show the same magnified region as in the middle panels for the ISH probe that detects ZFR transcripts (green) and MAPT transcripts (white) overlaid with the IHC signals from an anti-NeuN antibody stain (purple) and from an anti-S100β antibody stain (orange). The results show that the thalamus from an NHP treated with AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a MAPT-ZFR displayed high levels of ZFR expression and reduced MAPT expression in NeuN-positive neurons. Glial cells (labeled by the S100β stain) in the STAC-BBB/MAPT-ZFR (SEQ ID NO: 1509) treated animal retained MAPT transcripts due to ZFR expression being restricted to neurons by the hSYN1 promoter. At the single-cell level, ZFR transcript expression was strongly correlated with substantially reduced MAPT transcript levels in individual NeuN-positive neurons in the thalamus.

DETAILED DESCRIPTION OF THE INVENTION

[0035] 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: 1-1232 or 1525 and an expression construct encoding ZFP domains that target sites (*i.e.,* bind DNA sequences) in or near the human MAPT 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: 1-1232 or 1525 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 MAPT gene into mRNA. The

fusion proteins are called zinc finger protein-repressors (ZFRs) that target specifically the human MAPT 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 MAPT gene and a transcription repressor domain that reduces the transcription of the gene. Reducing the level of tau in neurons by introducing the ZFRs into the brain of a patient is expected to inhibit (*e.g.,* reduce or stop) the assembly of tau into aggregates and NFTs. Cell-to-cell propagation of tau aggregates will be reduced or prevented. The ZFRs can be used for the prevention and/or treatment of tauopathies. The ZFP domains disclosed herein may comprise a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4.

[0036] 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: 1-1232 or 1525 MAPT ZFP approach to tau inhibition has several advantages over the current approaches being tested by others, which include administration of (i) antisense oligonucleotides (ASOs) that bind tau mRNA and prevent its translation and (ii) immunotherapeutic anti-tau 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: 1235, see, FIG. 1(A)), 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: 1-1232 or 1525 MAPT ZFP approach only needs to engage the two alleles of the MAPT gene in the genome of each cell. By contrast, ASOs need to engage numerous copies of the *MAPT* 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: 1-1232 or 1525 MAPT ZFP approach 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: 1-1232 or 1525 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: 1-1232 or 1525 MAPT 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: 1-1232 or 1525 MAPT 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: 1-1232 or 1525 MAPT ZFP approach uses an AAV capsid protein comprising SEQ ID. NO: 1235 and an expression construct comprising a fusion protein, the fusion protein comprising a ZFP domain the ZFP domain comprising a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4. 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: 1-1232 or 1525 MAPT ZFP approach uses an AAV capsid protein comprising SEQ ID. NO: 1235 and an expression construct encoding a fusion protein comprising SEQ ID NO: 1509.

[0037] 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: 1-1232 or 1525 and an expression construct encoding a MAPT ZFP fusion protein is advantageous over the antibody approach because antibodies can only bind a subset of tau protein species or conformations. This may not be sufficient for a robust therapeutic effect. In contrast, ZFRs repress tau expression at the DNA level and lead to lower levels of all forms of tau, including different tau 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 tau, whereas ZFRs can reduce total tau levels inside the cell directly, thereby indirectly lowering extracellular tau 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: 1-1232 or 1525 (preferably SEQ ID NO: 71) and an expression construct encoding a MAPT ZFP fusion protein (preferably the fusion protein comprises SEQ ID NO: 1509) is expected to be more effective because tau 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: 1235 and an expression construct comprising a coding sequence for a fusion protein, the fusion protein comprising a ZFP domain the ZFP domain comprising a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4. In some embodiments, the composition for one-time delivery comprises and AAV capsid protein comprising SEQ ID NO: 1235 and an expression construct comprising a coding sequence for a fusion protein, the fusion protein comprising SEQ ID NO: 1509.

[0038] Tau modulators and methods and compositions for delivery thereof are disclosed in International Patent Publication WO 2018102665, the entirety of which is incorporated by reference herein (PCT Pat. App. No. PCT/US2017/064181). Zinc finger protein transcription factors for repressing tau expression are disclosed in International Patent Publication WO 21/151012, the entirety of which is incorporated by reference herein (PCT Pat. App. No. PCT/US2021/014780). Specifically, sections related to sequence listings, Targets of the ZFP Domains, Zinc Finger Protein Domains, Zinc Finger Protein Transcription Factors, Zinc Finger Repression Domains, Peptide Linkers, Expression of the ZFP-TFs 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 tau expression is disclosed in International Patent Publication WO 21/151012, 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 human *MAPT* gene. **FIG. 1(B)-(C)** illustrates the binding of a ZFP domain to a DNA sequence in the *MAPT* gene. The ZFP domain in the figure has six zinc fingers; however, as further described below, a ZFP domain that has fewer or more zinc fingers can also be used.

[0042] The human *MAPT* gene spans about 134 kb and has been mapped to chr17q21.31: 45,894,382 - 46,028,334 (GRCh38.p13). Its nucleotide sequence is available at GenBank accession number ENSG00000186868. The *MAPT* gene comprises 13-16 exons. Exons 1, 4, 5, 7, 9, 11 and 12 are constitutively expressed whereas exons 2, 3, and 10 can be present in tau protein species derived from alternatively spliced variants, leading to the presence of six different tau protein isoforms in the adult brain. Full-length human tau protein has the following sequence:

MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD
AGLKESPLQT PTEDGSEEPG SETSDAKSTP TAEDVTAPLV
DEGAPGKQAA AQPHTEIPEG TTAEEAGIGD TPSLEDEAAG
HVTQEPESGK VVQEGFLREP GPPGLSHQLM SGMPGAPLLP
EGPREATRQP SGTGPEDTEG GRHAPELLKH QLLGDLHQEG
PPLKGAGGKE RPGSKEEVDE DRDVDESSPQ DSPPSKASPA
QDGRPPQTAA REATSIPGFP AEGAIPLPVD FLSKVSTEIP
ASEPDGPSVG RAKGQDAPLE FTFHVEITPN VQKEQAHSEE
HLGRAAFPGA PGEGPEARGP SLGEDTKEAD LPEPSEKQPA
AAPRGKPVSR VPQLKARMVS KSKDGTGSDD KKAKTSTRSS
AKTLKNRPCL SPKHPTPGSS DPLIQPSSPA VCPEPPSSPK
YVSSVTSRTG SSGAKEMKLK GADGKTKIAT PRGAAPPGQK
GQANATRIPA KTPPAPKTPP SSGEPPKSGD RSGYSSPGSP
GTPGSRSRTP SLPTPPTREP KKVAVVRTPP KSPSSAKSRL
QTAPVPMPDL KNVKSKIGST ENLKHQPGGG KVQIINKKLD
LSNVQSKCGS KDNIKHVPGG GSVQIVYKPV DLSKVTSKCG
SLGNIHHKPG GGQVEVKSEK LDFKDRVQSK IGSLDNITHV
PGGGNKKIET HKLTFRENAK AKTDHGAEIV YKSPVVSGDT
SPRHLSNVSS TGSIDMVDSP QLATLADEVS
ASLAKQGL (SEQ ID NO: 1268; P10636)

[0043] The DNA-binding ZFP domains of the ZFRs direct the fusion proteins to a target region of the MAPT gene and bring the transcriptional repression domains of the fusion proteins to the target region. The repression domains recruit transcriptional co-repressor complexes to modify the chromatin into a non-permissive state for transcription by RNA Polymerase II. The target region for the ZFRs can be any suitable site in or near the MAPT 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 MAPT transcription start site (TSS) or a MAPT transcription regulatory element (*e.g.,* promoter, enhancer, RNA polymerase pause site, and the like).

[0044] 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, 16, 17, 18, 19, 20, 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 MAPT 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 12-20 (*e.g.,* 12-18, 15-19, 15, 18, or 19) bps in length and resides within 1500 bps upstream to 1000 bps downstream (*e.g.,* -1000 bps to +1000 bps, +750, or +500 bps) of the TSS.

[0045] In some embodiments, the engineered ZFPs bind to a target site (*i.e.,* Target Sequence) as shown in a single row of FIG. 2 or FIG. 4, preferably with no or little detectable off-target binding or activity, including contiguous or non-contiguous sequences within these target sites. In some embodiments, the target site comprises and/or is within any one of SEQ ID NOS: 1270-1321, and 1482. In some embodiments, the target site comprises and/or is within SEQ ID NO: 1311.

[0046] 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 off-target binding risk.

II. Zinc Finger Protein Domains

[0047] Zinc finger protein transcription factors for repressing tau expression is disclosed in International Patent Publication WO 21/151012, 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.

[0048] 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 and each finger binds from two to four base pairs of nucleotides, typically three or four base pairs of DNA (contiguous or noncontiguous). 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 .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).

[0049] A ZFP domain as described herein may be attached or fused to another molecule (*e.g.,* domain), 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 targeting the MAPT gene are shown in **FIG. 2** and **FIG. 4** and in International Patent Publication WO 21/151012, incorporated herein in its entirety by reference.

[0050] The ZFP domain of the engineered ZFP fusions 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.

[0051] In some embodiments, the engineered ZFPs comprise a DNA-binding recognition helix sequence having at least 4 of the amino acids of any recognition helix as shown in **FIG. 2** or **FIG. 4**. In some embodiments, the engineered ZFPs comprise a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4. In other embodiments, the engineered ZFPs comprise a DNA-binding recognition helix sequence shown in **FIG. 2** or **FIG. 4**. For example, an engineered ZFP may comprise the sequence of F1, F2, F3, F4, F5, or F6 as shown in **FIG. 2** or **FIG. 4**. In other embodiments, the engineered ZFPs comprise a fusion protein comprising SEQ ID NO: 1509.

[0052] In some embodiments, the engineered ZFPs comprise two adjacent DNA- binding recognition helix sequences shown in a single row of **FIG. 2** or **FIG. 4**.

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 **FIG. 2** or **FIG. 4**.

[0053] In some embodiments, the engineered ZFPs comprise the DNA-binding recognition helix sequences shown in a single row of **FIG. 2** or **FIG. 4**. For example, an engineered ZFP may comprise the sequences of F1, F2, F3, F4, F5, and F6 (*e.g.,* F1-F4, F1-F5, or F1-F6) as shown in a single row of **FIG. 2** or **FIG. 4**.

[0054] In some embodiments, an engineered ZFP described herein comprises the recognition helix and backbone portions of a sequence shown in a single row of **FIG. 3**. In some embodiments, an engineered ZFP described herein comprises the recognition helix and backbone portions of a sequence shown in a single row of **FIG. 3** 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 **FIG. 3**.

[0055] 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 (herein incorporated by reference in its entirety). The mutations include those made to residues in the ZFP backbone that can interact nonspecifically with phosphates on the DNA backbone but are not involved in nucleotide target specificity. 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 (-4), (-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 (-4), (-5), (-9) and/or (-14). In further embodiments, one or more zinc fingers in a multi-finger ZFP domain may comprise mutations at positions (-4), (-5), (-9) and/or (-14). In some embodiments, the amino acids at positions (-4), (-5), (-9) and/or (-14) (*e.g.,* an arginine (R) or lysine (K)) are mutated to an alanine (A), leucine (L), serine (S), aspartate (D), glutamate (E), tyrosine (Y), and/or glutamine (Q). In some embodiments, the R residue at position (-5) is mutated to Q. The symbol "^" in FIG. 4 indicates that the arginine (R) residue at the 4th position upstream of the 1st amino acid in the indicated recognition helix is changed to glutamine (Q). 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).

[0056] In some embodiments, the engineered ZFPs comprise a DNA-binding recognition helix sequence and associated backbone mutation as shown in FIG. 4. In some embodiments, the engineered ZFPs comprise the DNA-binding recognition helix sequences and associated backbone mutations as shown in a single row of FIG. 4. In some embodiments, the engineered ZFPs comprise the DNA-binding recognition helix sequences comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4. In some embodiments, the engineered ZFPs comprise the DNA-binding recognition helix sequences and associated backbone mutations as shown in SEQ ID NO: 1509 of FIG. 5.

[0057] In some embodiments, an engineered ZFP described herein comprises the recognition helix and backbone portions of a sequence shown in a single row of FIG. 5. In some embodiments, an engineered ZFP described herein comprises the recognition helix and backbone portions of the sequence shown in SEQ ID NO: 1509 of FIG. 5. In some embodiments, an engineered ZFP described herein comprises the recognition helix and backbone portions of a sequence shown in a single row of FIG. 5 as the sequence would appear following post-translational modification. In some embodiments, an engineered ZFP described herein comprises the recognition helix and backbone portions of the sequence shown in a single row of FIG. 5 as the sequence would appear following post-translational modification. In some embodiments, an engineered ZFP described herein comprises the recognition helix and backbone portions of the sequence shown in SEQ ID NO: 1509 in FIG. 5 as the sequence would appear following post-translational modification.

III. Zinc Finger Protein Transcription Factors

[0058] Zinc finger protein transcription factors for repressing tau expression is disclosed in International Patent Publication WO 21/151012, 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.

[0059] The ZFP domains described herein may be fused to a transcription factor. In some embodiments, the fusion proteins contain a DNA-binding zinc finger protein (ZFP) domain and a transcription factor domain (*i.e.*, ZFR). 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). In some embodiments, the

transcription factor may be a transcription repressor domain, wherein the SEQ ID NO: 1509 and repressor domains may be associated with each other by a direct peptidyl linkage or a peptide linker, or by dimerization. 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 can be associated with the ZFP (*e.g.,* SEQ ID NO: 1509) domain at any suitable position, including the C- or N-terminus of the ZFP domain.

[0060] In some embodiments, the ZFRs bind to their target with a KD of less than about 25 nM and repress transcription of a human *MAPT* gene by 20% or more (*e.g.,* by 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more). In some embodiments, two or more of the ZFRs are expressed in a cell to synergistically modulate *MAPT* expression in the cell (*see*, *e.g.,* U.S. Patent Application Publication NOS: 2020/0101133 (the entirety of which is incorporated by reference herein) and 2020/0109406 (the entirety of which is incorporated by reference herein). Such synergy ZFPs may be linked by a 2A linker peptide, *e.g.,* T2A GSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 1238). Thus, two or more ZFPs may be used concurrently in a patient, where the ZFPs bind to different target regions in the *MAPT* gene, so as to achieve optimal repression of *MAPT* expression. In some embodiments, the first ZFP comprises SEQ ID NO: 1509 and the second ZFP comprises the DNA-binding recognition helix sequences as shown in a single row of FIG 2 or FIG. 4 (but not ZFP ID 73133). In some embodiments, the first ZFP comprises SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4 and the second ZFP comprises the DNA-binding recognition helix sequences as shown in a single row of FIG 2 or FIG. 4 (but not ZFP ID 73133).

[0061] 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-ZFR, TF-ZFP-ZFP, ZFR-ZFP, or ZFR-ZFR (two ZFR fusion proteins are fused together via a linker).

[0062] 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.

[0063] In some embodiments, an engineered ZFR described herein binds to a target site as shown in a single row of FIG. 2 or FIG. 4, preferably with no or little detectable off-target binding or activity. In some embodiments, an engineered ZFP described herein binds to a target site as shown in SEQ ID NO: 1311, 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. In some embodiments, an engineered ZFR described herein comprises a DNA-binding recognition helix sequence shown in FIG. 2 or FIG. 4. In some embodiments, an engineered ZFP described herein comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4. In some embodiments, an engineered ZFR described herein comprises two adjacent DNA-binding recognition helix sequences shown in a single row of FIG. 2 or FIG. 4. In some embodiments, an engineered ZFR described herein comprises the DNA-binding recognition helix sequences shown in a single row of FIG. 2 or FIG. 4.

A. Transcription Repressor Domains

[0064] Zinc finger protein transcription factors for repressing tau expression is disclosed in International Patent Publication WO 21/151012, 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.

[0065] The ZFRs comprise an engineered ZFP domain as described herein and one or more transcription repressor domains that dampen the transcription activity of the *MAPT* 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 are the KRAB domain of KOX1 or ZIM3 (or any other KRAB domain containing protein. *See, e.g.,* Alerasool et al., *Nature Methods* (2020) 17:1093-6), 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), Rb, and MeCP2. *See, e.g.,* Bird et al., *Cell* (1999) 99:451-4; Tyler et al., *Cell* (1999) 99:443-6; Knoepfler et al., *Cell* (1999) 99:447-50; and Robertson et al., *Nature Genet.* (2000) 25:338-42. Additional exemplary repression domains include, but are not limited to, ROM2 and AtHD2A. *See, e.g.,* Chem et al., *Plant Cell* (1996) 8:305-21; and Wu et al., *Plant J.* (2000) 22:19-27. In one embodiment, SEQ ID NO: 1509 is operatively linked to one or more transcription repressor domains that dampen the transcription activity of the *MAPT* gene. In one embodiment, SEQ ID NO: 1509 and one or more transcription repressor domains are joined by a flexible linker. In one embodiment, SEQ ID NO: 1509 is joined by a flexible linker to a KRAB domain of KOX1 or ZIM3 (or any other KRAB domain containing protein). In some embodiments, the transcription repressor 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: 1239)

[0066] Variants of this KRAB sequence may also be used so long as they have the same or similar transcription repressor function.

B. Peptide Linkers

[0067] Zinc finger protein transcription factors for repressing tau expression is disclosed in International Patent Publication WO 21/151012, the entirety of which is incorporated by reference herein and specifically incorporated by reference in its entirety is the section related to Peptide linkers.

[0068] 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 200 amino acids (*e.g.,* 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acids). In one embodiment, the ZFP domain and/or the zinc fingers (SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 as shown in FIG. 4) within the ZFP domains and a transcription repressor domain are linked through a peptide linker, *e.g.,* a noncleavable peptide linker of about 5 to 200 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 (herein incorporated by reference in its entirety).

[0069] In some embodiments, the peptide linker is three to 20 amino acid residues in length and is rich in G and/or S. Non-limiting examples of such linkers are G4S-type linkers, *i.e.,* linkers containing one or more (*e.g.,* 2, 3, or 4) GGGGS (SEQ ID NO: 1269) motifs, or variations of the motif (such as ones that have one, two, or three amino acid insertions, deletions, and substitutions from the motif).

[0070] 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.

[0071] Non-limiting examples of linkers are DGGGS (SEQ ID NO: 1240), TGEKP (SEQ ID NO: 1241), LRQKDGERP (SEQ ID NO: 1242), GGRR (SEQ ID NO: 1243), GGRRGGGS (SEQ ID NO: 1244), LRQRDGERP (SEQ ID NO: 1245), LRQKDGGGSERP (SEQ ID NO: 1246), LRQKD(G3S)2 ERP (SEQ ID NO: 1247), TGSQKP (SEQ ID NO: 1248), LRQKDAARGS (SEQ ID NO: 1249), LRQKDAARGSGG (SEQ ID NO: 1250). Additional illustrative linkers for linking zinc fingers and/or for linking domains are listed in **Table 1**. The finger-finger linkers listed in **Table 1** include portions of backbone sequence, *e.g.,* FQ or FA.

[0072] **Table 1** 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 **FIG. 2** or **FIG. 4**. **Table 1** 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 FIG. 3 and FIG. 5.

Table 1

Linker Position	Linker Category	Exemplary Linker		
		Linker Code	Peptide sequence	SEQ ID NO:
Finger-Finger	No base skipping	0a	TGEKPFQ	1251
		0b	TGGQRPFQ	1252
		0c	TGSQKPFQ	1253
		0d	TGSQRPFQ	1254
		0f	TGEKPFA	1255
	1 base skipping	1a	TGGGGSQRPFQ	1256
		1b	TGGGGSQKPFQ	1257
		1c	THPRAPIPKPFQ	1258
		1d	TPNRRPAPKPFQ	1259
		1e	TVPRPTPPKPFQ	1260
		1f	TYPRPIAAKPFQ	1261
	2 base skipping	2a	TGGGGSGGSQRPFQ	1262
		2b	TGGGGSGGSQKPFQ	1263
		2d	TLAPRPYRPPKPFQ	1264
		2e	TPGGKSSRTDRNKPFQ	1265
		2f	TPNPHRRTDPSHKPFQ	1266
ZFP-functional domain (interdomain)	ZFR	C0	LRGSGG	1267
		C1	LRQKDAARGS	1249
		C1k	LRQKDAARGSGG	1250

[0073] In some embodiments, the engineered ZFPs described herein comprise two adjacent DNA-binding recognition helix sequences linked as shown in a single row of **FIG. 2** or **FIG. 4**. 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 **FIG. 2** or **FIG. 4**. In other embodiments, a different linker may be used from the same linker category. In some embodiments, the engineered ZFPs described herein comprise the DNA-binding recognition helix sequences linked as shown in a single row of **FIG. 2** or **FIG. 4**. For example, an engineered ZFP may comprise the linked sequences of F1-F4, F1-F5, or F1-F6 as shown in a single row of **FIG. 2** or **FIG. 4**. In other embodiments, one or more

different linkers may be used from the same linker category. In some embodiments, the engineered ZFPs described herein comprise the DNA-binding recognition helix sequences SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 linked as shown in FIG. 4 In some embodiments, the engineered ZFPs described herein comprise the DNA-binding recognition helix sequences linked as shown in SEQ ID NO: 1509. For example, an engineered ZFP may comprise the linked sequences of F1-F4, F1-F5, or F1-F6 for SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 as shown in FIG. 4. For example, an engineered ZFP may comprise the linked sequences of F1-F4, F1-F5, or F1-F6 as shown in SEQ ID NO: 1509.

[0074] In some embodiments, an engineered ZFR described herein comprises the recognition helix and linker portions of a sequence as shown in a single row of **FIG. 2** or **FIG. 4**. In some embodiments, an engineered ZFP described herein comprises the recognition helix and linker portions of SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4. In other embodiments, one or more different linkers may be used from the same linker category. In some embodiments, an engineered ZFR described herein comprises the recognition helix, backbone, and linker portions of a sequence as shown in a single row of **FIG. 3** or **FIG. 5**. In some embodiments, an engineered ZFP described herein comprises the recognition helix, backbone, and linker portions of a sequence as shown in SEQ ID NO: 1509. In other embodiments, one or more different linkers may be used from the same linker category. In some embodiments, an engineered ZFR described herein comprises an amino acid sequence as shown in a single row of **FIG. 3** or **FIG. 5**. In some embodiments, an engineered ZFR described herein comprises an amino acid sequence as shown in SEQ ID NO: 1509. In some embodiments, an engineered ZFP described herein comprises the recognition helix, backbone, and linker portions of a sequence shown in a single row of **FIG. 3** or **FIG. 5** as the sequence would appear following post-translational modification. In some embodiments, an engineered ZFP described herein comprises an amino acid sequence as shown in a single row of **FIG. 3** or **FIG. 5** as the sequence would appear following post-translational modification. In some embodiments, an engineered ZFP described herein comprises an amino acid sequence as shown in SEQ ID NO: 1509 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 **FIG. 3** or **FIG. 5**.

IV. Expression and Delivery of the ZFRs

[0075] Zinc finger protein transcription factors for repressing tau expression is disclosed in International Patent Publication WO 21/151012, 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 ZFP-TFs.

[0076] 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. Alternatively, the ZFR may be introduced to the patient through a nucleic acid expression vector/construct 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.

[0077] 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 Rous sarcoma virus (RSV) long terminal repeat (LTR) promoter (optionally with an RSV enhancer), a cytomegalovirus (CMV) promoter (optionally with a CMV enhancer), a CMV immediate early promoter, a simian virus 40 (SV40) promoter, a dihydrofolate reductase (DHFR) promoter, a 0-actin promoter, a phosphoglycerate kinase (PGK) promoter, an EFla promoter, a Moloney murine leukemia virus (MoMLV) LTR, a creatine kinase-based (CK6) promoter, a transthyretin promoter (TTR), a thymidine kinase (TK) promoter, a tetracycline responsive promoter (TRE), a hepatitis B Virus (HBV) promoter, a human al-antitrypsin (hAAT) promoter, chimeric liver-specific promoters (LSPs), an E2 factor (E2F) promoter, the human telomerase reverse transcriptase (hTERT) promoter, a CMV enhancer/chicken 0-actin/rabbit 0-globin promoter (CAG promoter; Niwa et al., *Gene* (1991) 108(2):193-9), and an RU-486-responsive promoter. Neuron-specific promoters such as a synapsin I promoter, a calcium/calmodulin-dependent protein kinase II (CamKII) promoter, a methyl CpG-binding protein 2 (MeCP2) promoter, a choline acetyltransferase (ChAT) promoter, a Calbindin (Calb) promoter, a CAMKII 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. Astrocyte-specific promoters such as the glial fibrillary acidic protein (GFAP) promoter or the aldehyde dehydrogenase 1

family, member L1 (Aldh1L1) promoter may also be used. Oligodendrocyte-specific promoters such as the Olig2 promoter may also be used. In addition, the promoter may include one or more self-regulating elements whereby the ZFR can bind to and repress its own expression level to a preset threshold. *See* U.S. Pat. 9,624,498.

[0078] For *in vivo* delivery of a *MAPT*-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 the ZFRs described herein (including but not limited to MAPT ZFR comprising SEQ ID NO: 1509), viral transduction is used using an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 (*i.e.,* CNSRCV300 in PCT Patent Application No. PCT/US24/29507 published as WO2024/238684 the entirety of which is incorporated by reference herein). An AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 is an engineered AAV capsid that crosses the blood brain barrier in non-human primates. AAV capsids were engineered to cross the blood brain barrier through insertion of a peptide sequence into variable region 8 of the AAV9 capsid. In some embodiments, the AAV capsid is assembled from the VP1, VP2, and/or VP3 sequences shown below in SEQ ID. NOs 1235, 1236 and 1237 encapsulating an expression construct encoding a MAPT ZFP (preferably a MAPT ZFP comprising SEQ ID NO: 1509). SEQ ID NOS: 1236 and 1237 are shorter versions of SEQ ID NO: 1235. SEQ ID NOS: 1235, 1236 and 1237 all comprise SEQ ID NO: 71. A skilled artisan understands that the full AAV capsid sequence is characterized by VP1, i.e., SEQ ID NOS: 1235, 1236 or 1237.

[0079] In some embodiments, targeting peptides (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) are 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 peptides (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) are introduced into VP1. In some embodiments, the targeting peptides (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) are introduced into VP2. In some embodiments, the targeting peptides (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) are introduced into VP3. In some embodiments, the targeting peptides (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) are introduced into VP1 and VP2. In some embodiments, the targeting peptides (SEQ ID NOS: 1-1232 or 1525, preferably

SEQ ID NO: 71) are introduced into VP1 and VP3. In some embodiments, the targeting peptides (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) are introduced into VP2 and VP3. In some embodiments, the targeting peptides (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) are introduced into VP1, VP2, and VP3. In some embodiments, the targeting peptide (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) is introduced at a single site in a capsid protein such as 1233 or 1234.

[0080] In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) 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: 1-1232 or 1525, preferably SEQ ID NO: 71) introduced into VP1. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) introduced into VP2. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) introduced into VP3. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) introduced into VP1 and VP2. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) introduced into VP1 and VP3. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) are introduced into VP2 and VP3. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) introduced into VP1, VP2, and VP3. In some embodiments, the AAV capsid protein comprises a targeting peptide (SEQ ID NOS: 1-1232 or 1525, preferably SEQ ID NO: 71) is introduced at a single site in a capsid protein such as 1233 or 1234.

[0081] In some embodiments, the AAV capsid protein comprises SEQ ID NO: 71 introduced into the AAV capsid proteins VP1, VP2 or VP3. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 71 introduced into two of the AAV capsid proteins VP1, VP2 or VP3 in any combination. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 71 introduced into the AAV capsid proteins VP1, VP2 and VP3. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 71 introduced into VP1 and not VP2 or VP3. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 71 introduced into VP2 and not VP1 or VP3. In some

embodiments, the AAV capsid protein comprises SEQ ID NO: 71 introduced into VP3 and not VP2 or VP1. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 71 introduced into VP1 and VP2 and not VP3. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 71 introduced into VP1 and VP3 and not VP2. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 71 introduced into VP2 and VP3 and not VP1. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 71 introduced into VP1, VP2, and VP3. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 71 is introduced at a single site in a capsid protein such as 1233 or 1234.

[0082] In some embodiments, the AAV capsid protein comprises SEQ ID NOS: 1235, 1236 or 1237. In some embodiments, the AAV capsid protein comprises SEQ ID NOS: 1235, 1236 and 1237. In some embodiments, the AAV capsid protein comprises SEQ ID NOS: 1235 or 1236 and not 1237. In some embodiments, the AAV capsid protein comprises SEQ ID NOS: 1235 or 1237 and not 1236. In some embodiments, the AAV capsid protein comprises SEQ ID NOS: 1236 or 1237 and not 1235. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 1235 and not 1236 or 1237. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 1236 and not 1235 or 1237. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 1237 and not 1236 or 1235.

[0083] In some embodiments, the composition comprises an AAV capsid protein, the AAV capsid protein comprising SEQ ID NOS: 1235, 1236 or 1237 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 microtubule-associated protein tau (*MAPT*) gene. In some embodiments, the composition comprises an AAV capsid protein the AVV capsid protein comprising SEQ ID NOS: 1235, 1236 and 1237 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 microtubule-associated protein tau (*MAPT*) gene. In some embodiments, the composition comprises an AAV capsid protein, the AAV capsid protein comprising SEQ ID NOS: 1235 or 1236 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 microtubule-associated protein tau (*MAPT*) gene. In some embodiments, the composition comprises an AAV capsid protein, the AAV capsid protein comprises SEQ ID NOS: 1235 or 1237 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 microtubule-associated protein tau (*MAPT*) gene. In some embodiments, the composition comprises an AAV capsid protein, the AAV protein comprises SEQ ID NOS: 1236 or 1237 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 microtubule-associated protein tau (*MAPT*) gene. In some embodiments, the composition comprises an AAV capsid protein, the AAV capsid protein comprises SEQ ID NO: 1235 and not 1236 or 1237 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 microtubule-associated protein tau (*MAPT*) gene. In some embodiments, the composition comprises an AAV capsid protein, the AAV capsid protein comprises SEQ ID NO: 1236 and not 1235 or 1237 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 microtubule-associated protein tau (*MAPT*) gene. In some embodiments, the composition comprises an AAV capsid protein, the AAV capsid protein comprises SEQ ID NO: 1237 and not 1236 or 1235 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 microtubule-associated protein tau (*MAPT*) gene.

[0084] 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: 1235 encapsulating an expression construct encoding MAPT ZFP (preferably a MAPT ZFP comprising SEQ ID NO: 1509). In some embodiments, an expression construct encoding MAPT ZFP (preferably a MAPT ZFP comprising SEQ ID NO: 1509) is packaged into an AAV capsid comprising an AAV

Capsid protein sequence from the group comprising, consisting, or consisting essentially of SEQ ID. NO: 1235, SEQ ID. NO: 1236, SEQ ID. NO: 1237 and combinations thereof encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509. In some embodiments, an expression construct encoding the ZFRs described herein is packaged into an AAV capsid comprising all of SEQ ID. NO: 1235, SEQ ID. NO: 1236, SEQ ID. NO: 1237. In some embodiments, an expression construct encoding the ZFRs described herein is packaged into an AAV capsid comprising one of SEQ ID. NO: 1235, SEQ ID. NO: 1236, SEQ ID. NO: 1237. In some embodiments, an expression construct encoding the ZFRs described herein is packaged into an AAV capsid comprising a plurality of SEQ ID. NO: 1235, SEQ ID. NO: 1236, SEQ ID. NO: 1237. 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: 1235. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1236. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1237. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NOS: 1235, 1236 and 1237. In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1235 and encapsulates an expression construct encoding a MAPT ZFP fusion protein (preferably SEQ ID NO: 1509). In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1236 and encapsulates an expression construct encoding a MAPT ZFP fusion protein (preferably SEQ ID NO: 1509). In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NO: 1237 and encapsulates an expression construct encoding a MAPT ZFP fusion protein (preferably SEQ ID NO: 1509). In some embodiments, the variant AAV capsid protein sequence is at least 80%, 85%, 90%, 95%, or 99% identical to a SEQ ID. NOS: 1235, 1236 and 1237 and encapsulates an expression construct encoding a MAPT ZFP fusion protein (preferably SEQ ID NO: 1509).

[0085] STAC-BBB VP1 capsid amino acid sequence:

MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLP
GYKYLGPGNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHAD
AEFQERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQS

PQEPDSSAGIGKSGAQPAKKRLNFGQTGDTESVPDPQPIGEPPAAPSGVGSLT
MASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVITTSTRTWALPTY
NNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQRLINN
NWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVL
GSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTG
NNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTL
KFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALN
GRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEE
EIKTTNPVATESYGQVATNHQSAYVNIMDDMDQAQTGWVQNQGILPGMVW
QDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPT
AFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVE
FAVNTEGVYSEPRPIGTRYLTRNL (SEQ ID NO: 1235)

[0086] STAC-BBB VP2 capsid amino acid sequence:

[0087] KTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGD
TESVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGNWHC
DSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWG
YFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIA
NNLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQA
VGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQ
YLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVT
QNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQ
GTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAYVNIMDDMDQ
AQTGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMK
HPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRW
NPEIQYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL (SEQ ID NO: 1236)

[0088] STAC-BBB VP3 capsid amino acid sequence

MASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVITTSTRTWALPTY
NNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQRLINN
NWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVL
GSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTG
NNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTL
KFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALN
GRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEE
EIKTTNPVATESYGQVATNHQSAYVNIMDDMDQAQTGWVQNQGILPGMVW

QDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPT AFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVE FAVNTEGVYSEPRPIGTRYLTRNL (SEQ ID NO: 1237)

[0089] Here, the novelty is the combination of a MAPT 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: 1-1232 or 1525. 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: 1-1232 or 1525 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: 1-1232 or 1525 (and in particular SEQ ID 1235) 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: 1235, 1236 , and 1237.

[0090] 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 SEQ ID NO: 71 encapsulating an expression construct encoding a MAPT ZFP fusion protein (preferably SEQ ID NO: 1509) 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 SEQ ID NO: 71 encapsulating an expression construct encoding a MAPT ZFP fusion protein (preferably SEQ ID NO: 1509) generally increases or enhances movement of the construct across the blood brain barrier compared to SEQ ID Nos 1233 or 1234. 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: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 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: 1233 or 1234). In particular examples, the increased movement of an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 across the blood brain barrier is observed in non-human primates in vivo. See Figs 6B-6H. An AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 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: 1-1232 or 1525 encapsulating an expression construct encoding a MAPT ZFP fusion protein (preferably SEQ ID NO: 1509) 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: 1-1232 or 1525 encapsulating an expression construct encoding a MAPT ZFP fusion protein (preferably SEQ ID NO: 1509) 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: 1-1232 or 1525 encapsulating an expression construct encoding a MAPT ZFP fusion protein (preferably SEQ ID NO: 1509) 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 a 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: 1-1232 or 1525 encapsulating an expression construct encoding a MAPT ZFP fusion protein (preferably SEQ ID NO: 1509) 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 1E14 vg/kg dose. In some embodiments, the subject is a mammal preferably a human.

[0091] An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NO: 1235 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 microtubule-associated protein tau (*MAPT*) gene wherein the ZFP domain comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4.

[0092] An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NO: 1236 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 microtubule-associated protein tau (*MAPT*) gene wherein the ZFP domain comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4.

[0093] An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NO: 1237 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 microtubule-associated protein tau (*MAPT*) gene wherein the ZFP domain comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4.

[0094] An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NOS: 1235, 1236, 1237 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 microtubule-associated protein tau (*MAPT*) gene wherein the ZFP domain comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4.

[0095] An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NOS: 1235, 1236, 1237 and combinations thereof and a

MAPT ZFR 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: 1235, 1236, 1237 and combinations thereof and a MAPT 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: 1235, 1236, 1237 and combinations thereof and a MAPT 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.

[0096] An embodiment comprises a composition comprising a capsid polypeptide having SEQ ID NOS: 1235, 1236, 1237 and combinations thereof encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 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: 1235, 1236, 1237 and combinations thereof encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 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: 1235, 1236, 1237 and combinations thereof encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 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.

[0097] 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: 1-1232 or 1525, 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: 1235, 1236, and/or 1237, 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 microtubule-associated protein tau (*MAPT*) gene. In some embodiments, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequence shown in a single row in FIGs 2 or 4. In some embodiments, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequences linked as shown in FIG. 3, or FIG. 5. In some embodiments, the ZFP domain comprising, consisting, or consisting essentially of an amino acid sequence selected from SEQ ID NOs: 1322-1429, 1430-1481 and 1483-1524. In some embodiments, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4. In some embodiments, the ZFP domain binds to a target sequence shown in FIG. 2 or FIG. 4. In some embodiments, the ZFP domain binds to a target sequence shown in SEQ ID NO: 1311. In some embodiments, the fusion protein comprising, consisting, or consisting essentially of a sequence shown in FIG. 3 or FIG. 5. In some embodiments, the fusion protein comprises a sequence having at least 80%, 85%, 90% or 99% identity to SEQ ID NO: 1509 In some embodiments, the fusion protein comprises, consists, or consists essentially of SEQ ID NO: 1509. In some embodiments, the AAV capsid protein comprises SEQ ID NO: 1235.

[0098] 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: 1-1232 or 1525 encapsulating an expression construct encoding a MAPT ZFP fusion protein (*e.g.,* the AAV capsid protein comprises SEQ ID NO: 1235 and the MAPT ZFP fusion protein comprises SEQ ID NO: 1509) 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, MAPT expression is reduced or stopped compared to prior to administration. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration, MAPT 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, MAPT 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, MAPT 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, MAPT 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, MAPT 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.

[0099] In some embodiments, the AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 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: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 is restricted to brain cell types following administration. In some embodiments, expression of an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 is not detected outside the brain following administration. In some embodiments, MAPT expression is reduced or stopped following administration of an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 compared to prior to administration only in brain cell types. In some embodiments, expression of an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 is restricted to CNS cell types. In some embodiments, MAPT expression following administration of an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 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: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 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: 1235 encapsulating

an expression construct encoding a fusion protein comprising SEQ ID NO: 1509. In some embodiments, 1-60 days, preferably 28, 29, or 30 days after administration of an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, 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: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, MAPT repression is dose-dependent. In some embodiments, the AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 dose is between 1E12 vg/kg to 5E14 vg/kg. In some embodiments, the AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 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: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, 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: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, MAPT 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: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, 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: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, MAPT 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: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, MAPT 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: 1235 encapsulating an expression construct encoding a fusion protein

comprising SEQ ID NO: 1509 has a hSYN1 promoter and S100β-positive glial cells do not express ZFP but neurons have ZFP expression.

[0100] Disclosed is a method of treating a subject with a dose of 1E14 vg/kg or between 1E12 vg/kg to 5E14 vg/kg, an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509. Following administration of an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, 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: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, 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 MAPT transcript levels in individual neurons is reduced compared to prior to administration. Following administration of an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, MAPT 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: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, MAPT 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: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 MAPT 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: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 has a hSYN1 promoter and S100β-positive glial cells do not express ZFP but neurons have ZFP expression.

[0101] V. CNS (Central Nervous System)-Targeting Molecules (Targeting Peptides)

[0102] 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).

[0103] 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.

[0104] In some embodiments, the CNS-targeting molecule comprises a sequence set forth in SEQ ID NO: 1-1232 or 1525. 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: 1-1232 or 1525. In some embodiments, the targeting peptide comprises the sequence set forth in SEQ ID NO: 71. 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: 71.

[0105] In some embodiments, the CNS-targeting molecule comprises variants of the amino acid sequences set forth in SEQ ID NO: 1-1232 or 1525. 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: 1-1232 or 1525. 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: 1-1232 or 1525. 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: 1-1232 or 1525. 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: 1-1232 or 1525. In some embodiments, the variant comprises any combination of the substitutions, deletions, or insertions described above.

[0106] 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: 1-1232 or 1525. 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: 1-1232 or 1525. 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: 1-1232 or 1525. 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: 1-1232 or 1525. 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: 1-1232 or 1525. In embodiments, the variant in the nucleotide sequence encodes any combination of the substitutions, deletions, or insertions described above.

[0107] 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: 1-1232 or 1525 are fused or conjugated to a small molecule, an antibody, scFV, ASO (antisense oligonucleotide), siRNA, lipid, polymer or recombinant protein. In some embodiments, SEQ ID NO: 71 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.

[0108] 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: 1233. In some embodiments, the AAV2 serotype comprises a variant of SEQ ID NO: 1233. In some embodiments, the AAV9 serotype comprises SEQ ID NO: 1234. In some embodiments, the AAV9 serotype comprises a variant of SEQ ID NO: 1234. In some embodiments, the AAV serotype comprises SEQ ID NOS: 1235, 1236, or 1237. In some embodiments, the AAV serotype comprises a variant of SEQ ID NOS: 1235, 1236, or 1237. 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: 1233 or 1234. 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: 1233 or 1234. 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: 1233 or 1234. 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: 1233 or 1234.

[0109] 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: 1233 or 1234 and at least 3, 4, 5, 6, 7, 8, 9 or all contiguous amino acids of any of SEQ ID NOS: 1-1232 or 1525. 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: 1233 or 1234 and at least 3, 4, 5, 6, 7, 8, 9 or all contiguous amino acids of any of SEQ ID NOS: 1-1232 or 1525.

[0110] 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: 1233. 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: 1234. 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: 1235. 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: 1236. 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: 1237. 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: 1235, 1236, and 1237. 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: 1234.

[0111] In embodiments, a variant refers to a variant in the nucleotide sequence that encodes the amino acid sequences set forth in SEQ ID NOs: 1235, 1236, or 1237. 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: 1235, 1236, or 1237. 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: 1233, or 1234. 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: 1233 or 1234. 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: 1233, or 1234.

[0112] 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: 1233 or 1234 and at least 3, 4, 5, 6, 7, 8, 9 or all contiguous amino acids of any of SEQ ID NOS: 1-1232 or 1525. 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: 1233 or 1234 and at least 3, 4, 5, 6, 7, 8, 9 or all contiguous amino acids of any of SEQ ID NOS: 1-1232 or 1525.

[0113] 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: 1233 and has at least 3, 4, 5, 6, 7, 8, 9 or all contiguous amino acids of any of SEQ ID NOS: 1-1232 or 1525. 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: 1234 and has at least 3, 4, 5, 6, 7, 8, 9 or all contiguous amino acids of any of SEQ ID NOS: 1-1232 or 1525. 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: 1235. 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: 1236. 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: 1237.

[0114] In some embodiments, any of the CNS-targeting molecules set forth in any of SEQ ID NO: 1-1232 or 1525 is inserted into an AAV. In some embodiments, any of the CNS-targeting molecules set forth in any of SEQ ID NO: 1-1232 or 1525 is inserted into AAV2. In some embodiments, AAV2 comprises the sequence set forth in SEQ ID NO: 1233. In some embodiments, SEQ ID NO: 71 is inserted into SEQ ID NO: 1233. SEQ ID NO: 1233 is AAV2 with an amino acid substitution. In some embodiments, any of the CNS-targeting molecules set forth in any of SEQ ID NO: 1-1232 or 1525 is inserted into AAV9. In some embodiments, AAV9 comprises the sequence set forth in SEQ ID NO: 1234. In some embodiments, SEQ ID NO: 71 is inserted into SEQ ID NO: 1234. SEQ ID NO: 1234 is AAV9. SEQ ID NO: 71 inserted into SEQ ID NO: 1234 can result in SEQ ID NOS: 1235, 1236, or 1237. In some embodiments, any of the CNS-targeting molecules set forth in any of SEQ ID NO: 1-1232 or 1525 is inserted into an AAV. In some embodiments, the AAV capsid protein comprises the sequence set forth in SEQ ID NOS: 1235, 1236, or 1237.

[0115] In some embodiments, insertion of SEQ ID NO: 71 into AAV9 results in the sequence set forth in SEQ ID NO: 1235 (CNSRCV300). In some embodiments, insertion of SEQ ID NO: 71 into AAV9 results in a variant sequence of SEQ ID NO: 1233 or 1234. 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: 1233 or 1234. 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: 1235, 1236, or 1237 or comprises SEQ ID NOS: 1235, 1236, or 1237. 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: 1235, 1236, or 1237 but is not SEQ ID NO: 1233 or 1234. 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: 1235, 1236, or 1237 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: 1235, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 71. In embodiments, the variant comprises at least 80%, 85%, 90%, 95%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 1236, or 1237, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 71.

[0116] 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: 1235, 1236, or 1237. 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: 1235, 1236, or 1237. 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: 1235, 1236, or 1237. 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: 1235, 1236, or 1237, and at least 3, 4, 5, 6, 7, 8, or 9 contiguous amino acids of SEQ ID NO: 71.

[0117] 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: 1233 or SEQ ID NO: 1234, 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: 1-1232 or 1525 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 microtubule-associated protein tau (*MAPT*) gene. In some embodiments, the ZFP domain comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4. In some embodiments, the fusion protein comprises SEQ ID NO: 1509.

[0118] 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: 1235, SEQ ID NO: 1236, or SEQ ID NO: 1237 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 microtubule-associated protein tau (*MAPT*) gene. In some embodiments, the ZFP domain comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4. In some embodiments, the fusion protein comprises SEQ ID NO: 1509.

[0119] AAV particles

[0120] 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.

[0121] Delivery of AAV Particles

[0122] 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.

[0123] 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.

[0124] 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.

[0125] 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.

[0126] AAV capsid proteins comprising CNS-targeting molecules (targeting peptides)

[0127] 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.

[0128] 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.

[0129] 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.

[0130] 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.

[0131] 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.

[0132] 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.

[0133] 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.

[0134] In some embodiments, the targeting peptide comprises an amino acid sequence of any sequence set forth in SEQ ID NO: 1-1232 or 1525. In some embodiments, the targeting peptide comprises the amino acid sequence set forth in SEQ ID NO: 71. 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: 1-1232 or 1525. 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: 71. 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.

[0135] 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: 1-1232 or 1525. 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: 71. 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: 1-1232 or 1525. 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: 71.

[0136] Insertion of Targeting Peptides into Capsid Proteins

[0137] 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.

[0138] In some embodiments, a peptide sequence that comprises any of the sequences set forth in SEQ ID NO: 1-1232 or 1525 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: 1-1232 or 1525. In some embodiments, disclosed is a peptide sequence that comprises the sequence set forth in SEQ ID NO: 71. 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 NO: 71.

[0139] 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: 1233. 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: 1233. 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: 1233. In some embodiments, the AAV capsid protein comprises the AAV9 serotype. In some embodiments, the AAV9 serotype includes the sequence of SEQ ID NO: 1234. 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: 1234. 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: 1234. 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: 1234. 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: 1-1232 or 1525. 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: 71. In some embodiments, insertion of the amino acid sequence set forth in SEQ ID NO: 71 into an AAV9 serotype results in the sequence set forth in SEQ ID NO: 1235 (CNSRCV300). In some embodiments, the AAV serotype comprises the sequence of SEQ ID NO: 1235. In some embodiments, the AAV serotype comprises the sequence of SEQ ID NOS: 1235, 1236, 1237 and combinations thereof. In some embodiments, insertion of the amino acid sequence set forth in SEQ ID NO: 71 into an AAV serotype results in the sequence set forth in SEQ ID NOS: 1235, 1236, or 1237.

[0140] 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: 1-1232 or 1525. In some embodiments, the targeting peptide comprises SEQ ID NO: 71.

[0141] 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: 1-1232 or 1525 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: 71 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: 1-1232 or 1525 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: 71 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: 1-1232 or 1525 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: 71 into the AAV capsid protein.

[0142] The peptide sequences listed in Table 2 were inserted into variable region 8 of AAV serotypes 2 and 9. In some embodiments, the peptides are inserted into AAV9 (SEQ ID 1234) 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 1 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 1233). For more detail see WO2024238684, incorporated by reference in its entirety.

[0143] Table 2.

Sequence ID	Peptide Sequence	Sequence ID	Peptide Sequence
Seq ID 1	NFRQSEMLN	Seq ID 616	MKVSAQVQS
Seq ID 2	QRNTDHITP	Seq ID 617	NVSQQKMVQ
Seq ID 3	MQFDPQKPD	Seq ID 618	RQQNQDKFS
Seq ID 4	TMSSNDNMR	Seq ID 619	MSSRTMEIH
Seq ID 5	VKESVQKAA	Seq ID 620	HEMIKQSGP
Seq ID 6	ILNVSKPDN	Seq ID 621	QDTDRAGKF
Seq ID 7	SQAAQLPTQ	Seq ID 622	KWEQRGMAH
Seq ID 8	QVMAIDAAI	Seq ID 623	TKQHTEMQQ
Seq ID 9	NDTARERAI	Seq ID 624	PMQALQNML

Seq ID 10	SMAAKVTAE
Seq ID 11	TLGHQHSPI
Seq ID 12	TVPSSGKQH
Seq ID 13	AMVQISTPL
Seq ID 14	VQVTGTVRF
Seq ID 15	MIAQKTVVH
Seq ID 16	NTHFQGQPP
Seq ID 17	MDMQATGKN
Seq ID 18	NLAHTRQPQ
Seq ID 19	LDIPPMNSS
Seq ID 20	QHSTPSWPS
Seq ID 21	AFTRHNGTGGKSIYGA
Seq ID 22	ARGARDEFT
Seq ID 23	RPSNDKAMM
Seq ID 24	AQVHMTMPQ
Seq ID 25	ARQTFQHHL
Seq ID 26	HQTDAPTDW
Seq ID 27	ANMISEPRISYGNDAA
Seq ID 28	IERWQAEII
Seq ID 29	VPHNGLHST
Seq ID 30	RIHDPSLVS
Seq ID 31	IPATHPQLM
Seq ID 32	ITTVKNVTV
Seq ID 33	DKSVKSTAI
Seq ID 34	IRTHEVPLQ
Seq ID 35	SPSSTSNNN
Seq ID 36	LSTTSQVHN
Seq ID 37	NMHNETSMT
Seq ID 38	NDSRKANIG
Seq ID 39	KPPNVQDAK
Seq ID 40	FQHMTPKPS
Seq ID 41	KQPSRLMEW
Seq ID 42	VYDQVKATH
Seq ID 43	SYRNQYDDQ
Seq ID 44	VQSTKQSVQ
Seq ID 45	HQGKVDAQN
Seq ID 46	IQGEIRVHT
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[0145] Seq ID 1234: AAV9

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[0146] Seq ID 1235: CNSRCV300

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VI. Pharmaceutical Applications

[0147] Zinc finger protein transcription factors for repressing tau expression is disclosed in International Patent Publication WO 21/151012, the entirety of which is incorporated by reference herein and specifically incorporated by reference in its entirety is the section related to Pharmaceutical Applications.

[0148] The compositions disclosed herein can be used to treat patients in need of downregulation of tau expression. The patients suffer from, or are at risk of developing, neurodegenerative diseases such as Alzheimer's disease, frontotemporal dementia, progressive supranuclear palsy, traumatic brain injury, seizure disorders, corticobasal degeneration, Parkinson's disease, dementia with Lewy bodies (DLB) and/or any other tauopathies. Patients at risk include those who are genetically predisposed, those who have suffered repeated brain injuries such as concussions, and those who have been exposed to environmental neurotoxins. Disclosed are methods of treating a neurological disease (*e.g.,* a tauopathy such as 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 MAPT expression) of the ZFR (*e.g.,* an rAAV vector expressing it). The term "treating" encompasses alleviation of symptoms, prevention of onset of symptoms, slowing of disease progression, improvement of quality of life, and increased survival.

[0149] The disclosure provides a pharmaceutical composition comprising a viral vector such as an rAAV whose recombinant genome comprises an expression cassette for the ZFRs. The pharmaceutical composition (*e.g.,* an artificial cerebrospinal fluid or aCSF), 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.

[0150] The cells targeted by the therapeutics of the 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.

[0151] The brain regions targeted by the 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. These regions can be reached directly through intrahippocampal injection, intracerebral injection, intra-cisterna magna (ICM) injection, or more generally through intraparenchymal injection, intrathalamic 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 intra- cisterna magna injection or intracerebroventricular 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.

[0152] 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 cerebrum, medulla, pons, cerebellum, thalamus, striatum, caudate, putamen, substantia nigra, midbrain, caudate, putamen, olfactory bulb, locus coeruleus, brain stem, globus pallidus, hippocampus, cerebral cortex, 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.

[0153] 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.

[0154] 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 (*e.g.,* 10^{10}-10^{15} Vg) 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 or near the ventricular region, as well as to the hippocampus, cerebral cortex, cerebellar lobule, cerebellum, cerebrum, medulla, pons, thalamus, striatum, caudate, putamen, substantia nigra, midbrain, caudate, putamen, olfactory bulb, locus coeruleus, brain stem, globus pallidus, intracranial cavity, meninges, dura mater, arachnoid mater, or pia mater of the brain, or other brain region. 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).

[0155] In some embodiments, following administration of an expression construct encoding MAPT ZFP encapsulated in an AAV capsid 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: 1-1232 or 1525, the production of tau protein in a subject's brain is reduced compared to before administration. In some embodiments, following administration of an expression construct encoding SEQ ID NO: 1509 encapsulated in an AAV capsid comprising SEQ ID. NO: 1235, the production of tau protein in a subject's brain is reduced compared to before administration.

[0156] In some embodiments, following administration of an expression construct encoding MAPT ZFP encapsulated in an AAV capsid 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: 1-1232 or 1525, the number of healthy neurons is higher compared to before administration. In some embodiments, following administration of an expression construct encoding SEQ ID NO: 1509 encapsulated in an AAV capsid comprising SEQ ID. NO: 1235, the number of healthy neurons is higher compared to before administration.

[0157] In some embodiments, the expression construct encoding a MAPT ZFP encapsulated into an AAV capsid comprising SEQ ID NO: 1235, see, FIG. 1(A), results in dose-dependent tau mRNA repression in one or more brain regions. In some embodiments, the expression construct encoding SEQ ID NO: 1509 encapsulated into an AAV capsid comprising SEQ ID NO: 1235 results in dose-dependent tau mRNA repression in a plurality of brain regions. In some embodiments, the expression construct encoding a MAPT ZFP encapsulated into an AAV capsid comprising SEQ ID NO: 1235, see, FIG. 1(A), results in dose-dependent tau mRNA repression in a plurality of brain regions, the brain regions being those affected in tauopathies. In some embodiments, the expression construct encoding SEQ ID NO: 1509 encapsulated into an AAV capsid comprising SEQ ID NO: 1235 results in dose-dependent tau mRNA repression in a plurality of brain regions, the brain regions being selected from the group comprising, consisting of, or consisting essentially of motor cortex, temporal cortex, precentral gyrus, cervical spinal cord, 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, the expression construct encoding a MAPT ZFP encapsulated into an AAV capsid comprising SEQ ID NO: 1235 results in dose-dependent tau mRNA repression in the motor cortex, temporal cortex, precentral gyrus, cervical spinal cord, thalamus, pons or combinations thereof. In some embodiments, the expression construct encoding SEQ ID NO: 1509 encapsulated into an AAV capsid comprising SEQ ID NO: 1235 results in dose-dependent tau mRNA repression in the motor cortex, temporal cortex, precentral gyrus, cervical spinal cord, thalamus, pons, or combinations thereof.

[0158] In some embodiments, the expression construct encoding a MAPT ZFP encapsulated into an AAV capsid comprising SEQ ID NO: 1235 results in dose-dependent tau mRNA repression in brain cells selected from the group comprising,

consisting of, or consisting essentially of the a motor neuron, a sensory neuron, a dopaminergic neuron, a cholinergic neuron, a glutamatergic neuron, a GABAergic neuron, a serotonergic neuron, a glial cell, an oligodendrocyte, an astrocyte, a pericyte, a Schwann cell, a microglial cell, a ependymal cell, a neuroepithelial cell and combinations thereof. In some embodiments, the expression construct encoding SEQ ID NO: 1509 encapsulated into an AAV capsid comprising SEQ ID NO: 1235 results in dose-dependent tau mRNA repression in cells selected from the group comprising, consisting of, or consisting essentially of the a motor neuron, a sensory neuron, a dopaminergic neuron, a cholinergic neuron, a glutamatergic neuron, a GABAergic neuron, a serotonergic neuron, a glial cell, an oligodendrocyte, an astrocyte, a pericyte, a Schwann cell, a microglial cell, a ependymal cell, a neuroepithelial cell and combinations thereof.

[0159] In some embodiments, the expression construct encoding a MAPT ZFP encapsulated into an AAV capsid comprising SEQ ID NO: 1235, see, FIG. 1(A), results tau repression at the single cell level. In some embodiments, the expression construct encoding SEQ ID NO: 1509 encapsulated into an AAV capsid comprising SEQ ID NO: 1235 results tau repression at the single cell level. In some embodiments, the expression construct encoding a MAPT ZFP encapsulated into an AAV capsid comprising SEQ ID NO: 1235, see, FIG. 1(A), results in stable tau repression. In some embodiments, the expression construct encoding SEQ ID NO: 1509 encapsulated into an AAV capsid comprising SEQ ID NO: 1235 results in stable tau repression.

[0160] 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.

[0161] While some embodiments comprise/include the disclosed features and may therefore include additional features not specifically described, other embodiments may be essentially free of or completely free of non-disclosed elements – that is, non-disclosed elements may optionally be essentially omitted or completely omitted.

[0162] 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.

VII. Exemplary Embodiments

[0163] Non-limiting exemplary embodiments of the disclosure are described below.

[0164] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 52288.

[0165] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 52389.

[0166] 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 SEQ ID NO: 71 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 FIG. 2, wherein the ZFP ID is 52364.

[0167] 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 SEQ ID NO: 71 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 FIG. 2, wherein the ZFP ID is 57890.

[0168] 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 SEQ ID NO: 71 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 FIG. 2, wherein the ZFP ID is 71214.

[0169] 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 SEQ ID NO: 71 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 FIG. 2, wherein the ZFP ID is 71218.

[0170] 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 SEQ ID NO: 71 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 FIG. 2, wherein the ZFP ID is 71225.

[0171] 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 SEQ ID NO: 71 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 FIG. 2, wherein the ZFP ID is 71227.

[0172] 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 SEQ ID NO: 71 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 FIG. 2, wherein the ZFP ID is 71249.

[0173] 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 SEQ ID NO: 71 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 FIG. 2, wherein the ZFP ID is 71304.

[0174] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71309.

[0175] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71310.

[0176] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71312.

[0177] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71341.

[0178] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71343.

[0179] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71345.

[0180] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71347.

[0181] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71351.

[0182] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71352.

[0183] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71357.

[0184] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71364.

[0185] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71366.

[0186] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71370.

[0187] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71373.

[0188] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71374.

[0189] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71377.

[0190] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71378.

[0191] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71385.

[0192] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71389.

[0193] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71391.

[0194] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71393.

[0195] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71395.

[0196] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71397.

[0197] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71398.

[0198] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71399.

[0199] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71400.

[0200] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71401.

[0201] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71402.

[0202] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71414.

[0203] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71420.

[0204] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71421.

[0205] 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71424.

[0206] Embodiment 43: 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71437.

[0207] Embodiment 44: 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71447.

[0208] Embodiment 45: 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71448.

[0209] Embodiment 46: 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71453.

[0210] Embodiment 47: 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71467.

[0211] Embodiment 48: 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71468.

[0212] Embodiment 49: 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71470.

[0213] Embodiment 50: 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71472.

[0214] Embodiment 51: 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71485.

[0215] Embodiment 52: 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 SEQ ID NO: 71 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 **FIG. 2**, wherein the ZFP ID is 71503.

[0216] Embodiment 53: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 65918.

[0217] Embodiment 54: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73015.

[0218] Embodiment 55: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73016.

[0219] Embodiment 56: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73017.

[0220] Embodiment 57: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73018.

[0221] Embodiment 58: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73019.

[0222] Embodiment 59: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73020.

[0223] Embodiment 60: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73021.

[0224] Embodiment 61: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73029.

[0225] Embodiment 62: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73030.

[0226] Embodiment 63: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73031.

[0227] Embodiment 64: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73032.

[0228] Embodiment 65: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73034.

[0229] Embodiment 66: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73035.

[0230] Embodiment 67: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73120.

[0231] Embodiment 68: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73121.

[0232] Embodiment 69: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73122.

[0233] Embodiment 70: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73123.

[0234] Embodiment 71: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73124.

[0235] Embodiment 72: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73125.

[0236] Embodiment 73: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73126.

[0237] Embodiment 74: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73127.

[0238] Embodiment 75: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73128.

[0239] Embodiment 76: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73129.

[0240] Embodiment 77: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73130.

[0241] Embodiment 78: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73131.

[0242] Embodiment 79: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73133.

[0243] Embodiment 80: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73190.

[0244] Embodiment 81: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73191.

[0245] Embodiment 82: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73192.

[0246] Embodiment 83: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73193.

[0247] Embodiment 84: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73194.

[0248] Embodiment 85: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73195.

[0249] Embodiment 86: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73196.

[0250] Embodiment 87: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73197.

[0251] Embodiment 88: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73198.

[0252] Embodiment 89: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73199.

[0253] Embodiment 90: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73200.

[0254] Embodiment 91: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73201.

[0255] Embodiment 92: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73202.

[0256] Embodiment 93: 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 SEQ ID NO: 71 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 **FIG. 4**, wherein the ZFP ID is 73203.

[0257] Embodiment 94: The composition of any one of embodiments 1-93, wherein the ZFR fusion protein comprises a transcription repressor domain.

[0258] Embodiment 95: The composition of embodiment 94, wherein transcription repressor domain comprises a KRAB domain.

[0259] Embodiment 96: The composition of embodiment 94, wherein transcription repressor domain comprises SEQ ID NO: 1239.

[0260] Embodiment 97: The composition of any one of embodiments 1-96, wherein the AAV capsid encapsulates the expression construct.

[0261] Embodiment 98: The composition of any one of embodiments 1-97, wherein the AAV capsid sequence is at least 80%, 85%, 90%, 95%, or 99% identical to SEQ ID NO: 1235, 1236, 1237 and combinations thereof.

[0262] Embodiment 99: The composition of any one of embodiments 1-98, wherein the ZFP domain binds to a target region of a human microtubule-associated protein tau (*MAPT*) gene.

[0263] Embodiment 100: The composition of any one of embodiments 1-99, wherein the ZFP domain binds to a target region of a human *MAPT* gene, wherein the target region is within 1.5 kb of a transcription start site (TSS) in the *MAPT* gene.

[0264] Embodiment 101: The composition of any one of embodiments 1-100, wherein the ZFP domain binds to a target region of a human *MAPT* gene wherein the target region is within 1000 bps upstream of the TSS, and/or within 500 bps downstream of the TSS of the *MAPT* gene.

[0265] Embodiment 102: The composition of any one of embodiments 99-101, wherein the fusion protein represses expression of the *MAPT* 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.

[0266] Embodiment 103: The composition of any one of embodiments 94-96, wherein the DNA-binding zinc finger recognition helix is linked to the transcription repressor through a peptide linker.

[0267] Embodiment 104: The composition of any one of embodiments 1-103, wherein the DNA-binding zinc finger recognition helix binds to binds to a target sequence shown in FIG. 2 or FIG. 4.

[0268] Embodiment 105: 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 SEQ ID NO: 71 ; and 2) 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 microtubule-associated protein tau (*MAPT*) gene, wherein the ZFP domain of the fusion protein comprises four, five, or six zinc fingers; binds to a target sequence shown in FIG. 2 or FIG. 4; comprises the DNA-binding recognition helix sequences of a ZFP transcription factor shown in FIG. 3 or FIG. 5; comprises the DNA-binding recognition helix sequences linked as shown in FIG. 2, FIG. 3, FIG. 4, or FIG. 5; and/or comprises an amino acid sequence selected from SEQ ID NOs: 1322-1429, 1430-1481 and 1483-1524.

[0269] Embodiment 106: A composition comprising an AAV capsid polypeptide, comprising a peptide modification relative to the AAV9 capsid polypeptide set forth in SEQ ID NO: 1234, 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: 1234, and comprises the sequence set forth in any one of SEQ ID Nos:1-1232 or 1525; 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: 1234 and 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 microtubule-associated protein tau (*MAPT*) gene wherein the ZFP domain comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4.

[0270] Embodiment 107: A pharmaceutical composition comprising the composition of any one of embodiments 1-106, and a pharmaceutically acceptable carrier.

[0271] Embodiment 108: A host cell comprising the composition of any one of embodiments 1-106.

[0272] Embodiment 109: A host cell comprising the composition of any one of embodiments 1-106 wherein the host cell is a human cell.

[0273] Embodiment 110: A host cell comprising the composition of any one of embodiments 1-106 wherein the host cell is a brain cell.

[0274] Embodiment 111: A host cell comprising the composition of any one of embodiments 1-106 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.

[0275] Embodiment 112: A method of inhibiting expression of tau in a human brain cell, comprising introducing into a cell a composition of any one of embodiments 1-106.

[0276] Embodiment 113: A method of inhibiting expression of tau in a human brain cell, comprising introducing into a subject in need thereof a composition of any one of embodiments 1-106.

[0277] Embodiment 114: The method of embodiment 112-113 wherein the AAV capsid penetrates across the blood brain barrier.

[0278] Embodiment 115: The method of any one of embodiments 112-114 wherein the patient shows ZFP expression.

[0279] Embodiment 116: The method of any one of embodiments 112-115 wherein the patient shows ZFP expression in the brain and/or spinal cord.

[0280] Embodiment 117: The method of any one of embodiments 112-116 wherein the patient does not show ZFP expression outside the brain and/or spinal cord.

[0281] Embodiment 118: The method of any one of embodiments 112-117 wherein the patient shows ZFP expression in ChAT-positive motor neurons in the spinal cord.

[0282] Embodiment 119: The method of any one of embodiments 112-118 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.

[0283] Embodiment 120: The method of any one of embodiments 112-119 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.

[0284] Embodiment 121: The method of any one of embodiments 112-120 wherein the patient shows ZFP expression in thalamus, pons, cervical spinal cord, precentral gyrus, and temporal cortex.

[0285] Embodiment 122: The method of any one of embodiments 112-121 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.

[0286] Embodiment 123: The method of any one of embodiments 112-122 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.

[0287] Embodiment 124: The method of any one of embodiments 112-123 wherein in S100β-positive glial cells, ZFP expression is not detected.

[0288] Embodiment 125: The method of any one of embodiments 112-125 wherein ZFP expression is restricted to neurons by a hSYN1 promotor.

[0289] Embodiment 126: The method of any one of embodiments 112-125 wherein ZFP expression in S100β-positive glial cells is the same as prior to the introducing of the composition.

[0290] Embodiment 127: The method of any one of embodiments 112-126 wherein the patient shows tau repression compared to prior to the introducing of the composition.

[0291] Embodiment 128: The method of any one of embodiments 112-127 wherein the patient shows tau repression in the brain and/or spinal cord compared to prior to the introducing of the composition.

[0292] Embodiment 129: The method of any one of embodiments 112-128 wherein the patient shows tau repression in ChAT-positive motor neurons in the spinal cord.

[0293] Embodiment 130: The method of any one of embodiments 112-129 wherein the patient shows tau 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.

[0294] Embodiment 131: The method of any one of embodiments 112-130 wherein the patient shows tau 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.

[0295] Embodiment 132: The method of any one of embodiments 112-131wherein the patient shows tau 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.

[0296] Embodiment 133: The method of any one of embodiments 112-132 wherein the patient shows tau 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.

[0297] Embodiment 134: The method of any one of embodiments 112-133 wherein the patient shows tau repression in the brain and spinal cord.

[0298] Embodiment 135: The method of any one of embodiments 112-134 wherein the patient shows tau repression in a plurality of NeuN-positive cells wherein ZFP expression is below the limit of detection of an In Situ Hybridization assay.

[0299] Embodiment 136: The method of any one of embodiments 112-135 wherein the subject is a human.

[0300] Embodiment 137: The method of any one of embodiments 112-137 wherein the patient shows tau 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.

[0301] Embodiment 138: A method of inhibiting expression of tau in a human brain cell, comprising providing to a cell a composition of any one of claims 1-106 or a pharmaceutical composition according to claim 107, thereby inhibiting the expression of tau in the cell.

[0302] Embodiment 139: The method of embodiment 138, wherein the human brain cell is a neuron, a glial cell, an ependymal cell, a neuroepithelial cell, an endothelial cell, or an oligodendrocyte.

[0303] Embodiment 140: The method of any one of embodiments 138-139, 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.

[0304] Embodiment 141: The method of any one of embodiments 138-140, comprising introducing into the cell the expression construct that expresses the fusion protein.

[0305] Embodiment 142: A method of treating a tauopathy in a patient in need thereof, comprising administering to the patient a composition of any one of embodiments 1-106 or a pharmaceutical of embodiment 107.

[0306] Embodiment 143: The method of embodiment 142, wherein the composition is introduced to the patient via an intravenous, intrathecal, intracerebral, intracerebroventricular, intra-cisternal magna, intrahippocampal, intrathalamic, or intraparenchymal route.

[0307] Embodiment 144: The method of embodiment 142 or 143, 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).

[0308] Embodiment 145: The method of embodiment 142, 143 or 144, wherein the composition is introduced to the patient at a concentration of 1E14 vg/kg or between 1E12 vg/kg to 5E14 vg/kg.

[0309] Embodiment 146: The method of any one of embodiments 112-142, 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.

[0310] Embodiment 147: The method of any one of embodiments 112-142 or 142, 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.

[0311] Embodiment 148: The composition of any one of embodiments 1-106, 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 1-106.

[0312] Embodiment 149: The composition of any one of embodiments 1-106, the pharmaceutical composition of embodiment 107 or the host cell of embodiments 108-111 for use in a method of inhibiting expression of tau in a human brain cell.

[0313] Embodiment 150: Use of composition of any one of embodiments 1-106, the pharmaceutical composition of embodiment 107 or the host cell of embodiments 108-111, for the manufacture of a medicament for inhibiting expression of tau in a human brain cell and/or treating a tauopathy in a patient.

[0314] Embodiment 151: Use of composition of any one of embodiments 1-106, 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 112-144.

[0315] Non-limiting exemplary embodiments of the disclosure are further described below.

[0316] 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: 1234, 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: 1-1232 or 1525, 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: 1234 and 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 microtubule-associated protein tau (*MAPT*) gene wherein the ZFP domain comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4.

[0317] 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: 1234.

[0318] 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: 1234 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: 1234.

[0319] 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: 1234.

[0320] Embodiment 5: The composition of embodiment 1, 2, 3 or 4, wherein the peptide modification comprises an amino acid insertion of SEQ ID NO:71.

[0321] Embodiment 6: The composition of embodiment 1, 2, 3, 4 or 5, wherein the ZFP domain comprises a DNA-binding recognition helix sequence as shown in SEQ ID NO: 1509.

[0322] 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: 1235; b) a VP2 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1236; c) a VP3 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1237, 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, wherein the ZFP domain binds to a target region of a human microtubule-associated protein tau (*MAPT*) gene wherein the ZFP domain comprises a DNA-binding

recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4.

[0323] Embodiment 8: The composition of embodiment 7, wherein the ZFP domain comprises a DNA-binding recognition helix sequence as shown in SEQ ID NO: 1509.

[0324] 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: 1234.

[0325] 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: 1234.

[0326] Embodiment 11: A composition of any one of embodiments 1-10 wherein the capsid encapsulates the fusion protein.

[0327] Embodiment 12: A composition comprising a vector comprising a nucleic acid molecule encoding SEQ ID NOS: 1235, 1236, or 1237 and 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 microtubule-associated protein tau (*MAPT*) gene wherein the ZFP domain comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4.

[0328] Embodiment 13: A composition comprising a vector comprising a nucleic acid molecule encoding SEQ ID Nos: 1235, 1236, or 1237 and 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 microtubule-associated protein tau (*MAPT*) gene wherein the ZFP domain comprises a DNA-binding recognition helix sequence as shown in SEQ ID NO: 1509.

[0329] Embodiment 14: A host cell, comprising the composition of any one of embodiments 1-13.

[0330] 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.

[0331] Embodiment 16: The method of embodiment 15, wherein the host cell is a human brain cell.

[0332] Embodiment 17: The method of embodiment 15 or 16, wherein contacting a host cell with the composition comprises administering the composition to a subject.

[0333] Embodiment 18: The method of embodiment 17, wherein administration of the composition to the subject effects treatment of a tauopathy-associated disease or condition.

[0334] Embodiment 19: The method of any one of embodiments 15-19, wherein the method is in vitro or ex vivo.

[0335] 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.

[0336] Non-limiting exemplary embodiments of the disclosure are further described below.

[0337] 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: 1234, 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: 1234, and comprises the sequence set forth in any one of SEQ ID Nos:1-1232 or 1525; 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: 1234 and 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 microtubule-associated protein tau (*MAPT*) gene wherein the ZFP domain comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4 for inhibiting expression of tau in a human brain cell and/or treating a tauopathy in a patient.

[0338] 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: 1234.

[0339] 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: 1234 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: 1234.

[0340] 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: 1234.

[0341] 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:71.

[0342] Embodiment 6: Use of the composition of embodiment 1, 2, 3, 4 or 5, wherein the ZFP domain comprises a DNA-binding recognition helix sequence as shown in SEQ ID NO: 1509.

[0343] 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: 1235; b) a VP2 protein comprising the sequence of amino acids set forth in SEQ ID NO:1236; c) a VP3 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1237, 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, wherein the ZFP domain binds to a target region of a human microtubule-associated protein tau (*MAPT*) gene wherein the ZFP domain comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4 for inhibiting expression of tau in a human brain cell and/or treating a tauopathy in a patient.

[0344] Embodiment 8: Use of the composition of embodiment 7, wherein the ZFP domain comprises a DNA-binding recognition helix sequence as shown in SEQ ID NO: 1509.

[0345] 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: 1234.

[0346] 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: 1234.

[0347] Embodiment 11: Use of the composition of any one of embodiments 1-10 wherein the capsid encapsulates the fusion protein.

[0348] Embodiment 12: Use of a composition comprising a vector comprising a nucleic acid encoding SEQ ID NOS: 1235, 1236, or 1237 and 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 microtubule-associated protein tau (*MAPT*) gene wherein the ZFP domain comprises a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4.

[0349] Embodiment 13: Use of a composition comprising a vector comprising a nucleic acid encoding SEQ ID NOS: 1235, 1236, or 1237 and 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 microtubule-associated protein tau (*MAPT*) gene wherein the ZFP domain comprises a DNA-binding recognition helix sequence as shown in SEQ ID NO: 1509.

[0350] 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.

[0351] 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.

[0352] 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.

[0353] 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.

[0354] Embodiment 18: Use of the composition of any one of embodiments 1-17, wherein the method is in vitro or ex vivo.

EXAMPLES

[0355] Example 1: Production of STAC-BBB harboring a MAPT-targeted ZFR

[0356] *MAPT*-targeted ZFRs were designed and made as described in International Patent Publication WO 21/151012, the entirety of which is incorporated by reference herein (PCT Pat. App. No. PCT/US2021/014780). To evaluate the performance of STAC-BBB to deliver a ZFR transgene cargo across the blood-brain barrier for the potential treatment of tauopathies, a ZFP targeting a conserved DNA sequence in the human and nonhuman primate *MAPT* gene was fused to the KRAB repression domain derived from the human *ZNF10* gene. (FIG. 1(A)-(C)). This ZFR (ZFP ID 73133) was cloned downstream of the hSYN1 promoter to restrict the expression of the ZFR to neurons, the target cell type for *MAPT* lowering in tauopathy disorders. This transgene cassette was flanked by AAV2 inverted terminal repeat sequences.

[0357] 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 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 were transfected into the cells using calcium phosphate as described in PCT Patent Application No. PCT/US24/29507 (published as WO2024/238684), incorporated herein in its entirety by reference. 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.

[0358] 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 formulated in aliquots and stored at -80°C until use.

[0359] Example 2: Widespread ZFR expression and MAPT repression in the nonhuman primate brain following delivery of an AAV Capsid Protein Comprising SEQ ID. NO: 1235 Encapsulating an Expression Construct Encoding A MAPT ZFP Fusion Protein

[0360] Adult cynomolgus macaques were injected intravenously with a single bolus of either formulation buffer (vehicle), a 5E12 vg/kg dose of an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, a 2E13 vg/kg dose of an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, or a 1E14 vg/kg dose of an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509. Animals were euthanized 28 days after test article administration. Brains were sectioned into 4mm coronal slices. (FIG. 6A). From each animal, a total of 220 punch biopsies (2mm in diameter) were collected from the right brain hemisphere across 10 brain levels spanning 35 regions for bulk RNA expression analysis. [084] Brain punches were incubated in RNALater for 24 hours at 4°C. 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 ZFR and nonhuman primate MAPT transcripts. ZFR transcript levels were normalized to the input RNA quantity used to produce the cDNA for a qPCR reaction. MAPT gene expression levels were normalized to the mean of the expression levels of the housekeeping genes ATP5B and EIF4A2. The normalized MAPT value for each punch for a given brain region and location was scaled to the average value of the control-treated animals. For each region, at least 2 punches were analyzed per animal.

[0361] ZFR expression values for >100 punches spanning 7 coronal levels across the rostrocaudal axis for a representative animal from each dose level are shown in FIG. 6B. Average ZFR expression and MAPT expression values for the 3 animals per dose group are shown in FIG. 6C. The data show treatment with an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion

protein comprising SEQ ID NO: 1509 resulted in efficient, widespread MAPT ZFR expression that was detected in punches at all levels and brain regions analyzed. ZFR expression increased in a dose-dependent manner, and correspondingly resulted in a dose-dependent decrease in MAPT levels in exemplary brain regions, including the pons and thalamus. The highest ZFR expression and MAPT repression was observed at the 1E14 vg/kg dose.

[0362] Example 3: Neuronal ZFR expression throughout the CNS and single-cell reduction of MAPT transcripts mediated by an AAV capsid protein comprising SEQ ID. NO: 1235

[0363] For the left brain hemisphere, 4mm coronal sections were fixed in 10% NBF at RT for approximately 24 hours. After fixation, sections were transferred to 70% ethanol, embedded into paraffin blocks and sectioned for multiplexed RNAscope-immunohistochemistry single-cell analysis. A multiplexed approach was used that combined in situ hybridization (ISH) to detect mRNA (using RNAscope probes specific for the ZFR or nonhuman primate MAPT transcripts) and immunohistochemistry (IHC) to label neurons (NeuN), motor neurons (ChAT), or glial cells (S100β).

[0364] Results for one of the animals treated with the 1E14 vg/kg dose of an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509 are shown for exemplary CNS regions, including the pons region of the brainstem (FIG. 6D), cervical level of the spinal cord (FIG. 6E), precentral gyrus region of the motor cortex (FIG. 6F), temporal cortex (FIG. 6G), and thalamus (FIG. 6H).

[0365] The results show that ZFR expression was observed in NeuN-positive neurons across all brain regions analyzed and in ChAT-positive motor neurons in the spinal cord. Expression of the ZFR was associated with substantial reduction of MAPT transcript levels in individual neurons. For the animal only treated with an AAV capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509, MAPT transcripts were reduced or absent in many NeuN-positive cells despite undetectable ZFR signal, indicating that levels of ZFR transcripts that are below the limit of detection of the ISH assay were still sufficient to mediate in MAPT reduction.

[0366] In S100β-positive glial cells, ZFR expression was not detected due to the hSYN1 promoter restricting expression to neurons. In these cells, there was no difference in MAPT transcript levels between the vehicle and treatment with an AAV

capsid protein comprising SEQ ID. NO: 1235 encapsulating an expression construct encoding a fusion protein comprising SEQ ID NO: 1509.

[0367] Collectively, the results show that an AAV capsid protein comprising SEQ ID. NO: 1235 mediates efficient and widespread delivery of a MAPT-ZFR cargo to the entire brain and spinal cord, resulting in lowering of MAPT at the bulk tissue and single-neuron level.

CLAIMS

1. 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 SEQ ID NO: 71; 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, wherein the ZFP domain binds to a target region of a human microtubule-associated protein tau (*MAPT*) gene.

2. The composition of claim 1, wherein the AAV capsid protein encapsulates the expression construct.

3. The composition of any one of the preceding claims, 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: 71 inserted into SEQ ID. NO: 1234, optionally wherein the insertion is between amino acids 587 and 590.

4. The composition of any one of the preceding claims, the AAV capsid protein comprising one or more amino acid sequences selected from the group comprising, consisting, or consisting essentially of SEQ ID NO: 1235, 1236, or 1237.

5. The composition of any one of the preceding claims, the AAV capsid protein comprising a VP1 protein comprising a sequence of amino acids set forth in SEQ ID NO: 1235; a VP2 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1236; and a VP3 protein comprising the sequence of amino acids set forth in SEQ ID NO: 1237.

6. The composition of any one of the preceding claims, the AAV capsid protein comprising the amino acid sequence having at least 80%, 85%, 90%, 95%, 99% or 100% identity to SEQ ID NO: 1235.

7. The composition of any one of the preceding claims, the AAV capsid protein comprising the amino acid sequence having at least 80%, 85%, 90%, 95%, 99% or 100% identity to SEQ ID NO: 1236 or to SEQ ID NO: 1237.

8. The composition of any one of the preceding claims, wherein the target region

is within 1.5 kb of a transcription start site (TSS) in the *MAPT* gene.

9. The composition of any one of the preceding claims, wherein the target region is within 1000 bps upstream of the TSS, and/or within 500 bps downstream of the TSS of the *MAPT* gene.

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

11. The composition of any one of the preceding claims, the transcription repressor domain comprises a KRAB domain, wherein the KRAB domain optionally is from a human KOX1 protein.

12. The composition of any one of the preceding claims, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequence shown in a single row in FIGs 2 or 4.

13. The composition of any one of the preceding claims 1-11, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequences linked as shown in FIG. 3, or FIG. 5.

14. The composition of any one of the preceding claims, the ZFP domain comprising, consisting, or consisting essentially of an amino acid sequence selected from SEQ ID NOs: 1322-1429, 1430-1481 and 1483-1524.

15. The composition of any one of the preceding claims, the ZFP domain comprising, consisting, or consisting essentially of a DNA-binding recognition helix sequence comprising SEQ ID NOS: 1322, 1379, 1346, 1378, 1394, 1409 ordered as shown in FIG. 4.

16. The composition of any one of the preceding claims, the ZFP domain binds to a target sequence shown in FIG. 2 or FIG. 4.

17. The composition of any one of claims 1-16, the ZFP domain binds to a target sequence shown in SEQ ID NO: 1311.

18. The composition of any one of the preceding claims, the fusion protein

comprising, consisting, or consisting essentially of a sequence shown in FIG. 3 or FIG. 5.

19. The composition of any one of the preceding claims, the fusion protein comprising a sequence having at least 80%, 85%, 90% or 99% identity to SEQ ID NO: 1509.

20. The composition of any one of the preceding claims, the fusion protein comprising SEQ ID NO: 1509.

21. The composition of any one of the preceding claims, the fusion protein under control of the human Synapsin1 (hSYN1) promoter.

22. The composition of any one of the preceding claims, wherein the fusion protein is expressed in brain cells or spinal cord cells of a patient that has been injected with the composition.

23. The composition of claim 22, wherein the fusion protein is expressed in one or in more than one brain cell of the patient wherein the brain cell is 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.

24. The composition of any one of claims 22-23, wherein the fusion protein is expressed in one or in more than one brain region of the patient 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 or combinations thereof.

25. The composition of any one of claims 22-24, wherein the fusion protein is expressed in one or in more than one brain region of the patient wherein the brain region is selected from the group comprising, consisting, or consisting essentially of the brainstem, spinal cord, motor cortex, temporal cortex, thalamus or combinations thereof.

26. The composition of any one of claims 22-25, wherein the fusion protein is expressed in the pons region of the brainstem, cervical level of the spinal cord, precentral gyrus region of the motor cortex, temporal cortex, and thalamus.

27. The composition of any one of claims 22-26, wherein the fusion protein is not expressed in S100β-positive glial cells.

28. The composition of any one of claims 22-27, wherein the fusion protein is expressed in ChAT-positive motor neurons in the spinal cord.

29. The composition of any one of claims 22-27, wherein the fusion protein is administered to a patient via a one-time intravenous, intrathecal, intracerebral, intracerebroventricular, intra-cisternal magna, intrahippocampal, intrathalamic, or intraparenchymal administration.

30. The composition of any one of claims 22-28, wherein the fusion protein is administered at a dose between 1E12 vg/kg to 5E14 vg/kg or therapeutically effective amount.

31. A nucleic acid construct encoding the expression construct of any one of claims 1, or 8-21.

32. A pharmaceutical composition comprising the composition of any one of claims 1-21 or the nucleic acid construct of claim 31, and a pharmaceutically acceptable carrier.

33. A method of inhibiting expression of tau in a human brain cell, comprising providing to a cell the composition of any one of claims 1-21 or the pharmaceutical composition according to claim 32, thereby inhibiting the expression of tau in the cell.

34. A method of inhibiting expression of tau in a human brain cell, comprising introducing into a patient in need thereof the composition of any one of claims 1-21 or the pharmaceutical composition according to claim 32, thereby inhibiting the expression of tau in the patient.

35. The method of claim 33 or 34, wherein the human brain cell is selected from the group comprising, consisting, or consisting essentially of a neuron, a glial cell, an

ependymal cell, a neuroepithelial cell, an endothelial cell, or an oligodendrocyte.

36. The method of any one of claims 33 through 35, wherein the human brain 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.

37. A method of treating a tauopathy in a patient in need thereof, comprising introducing into a patient in need thereof the composition of any one of claims 1-21 or the pharmaceutical composition of claim 32.

38. The method of any one of claims 33 through 37, wherein the composition is introduced to the patient via an intravenous, intrathecal, intracerebral, intracerebroventricular, intra-cisternal magna, intrahippocampal, intrathalamic, or intraparenchymal route.

39. The method of any one of claims 33 through 38, wherein the composition is introduced to the patient at a dose between 1E12 vg/kg to 5E14 vg/kg, 1E14 vg/kg or therapeutically effective amount.

40. The method of any one of claims 33 through 39, wherein the AAV capsid penetrates across the patient blood brain barrier.

41. The method of any one of claims 33 through 40, wherein the patient has fusion protein expression.

42. The method of any one of claims 33 through 41, wherein the patient has fusion protein expression in brain cells and/or spinal cord cells.

43. The method of any one of claims 33 through 42, wherein the patient has fusion protein expression in one or in more than one brain cell wherein the brain cell is 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.

44. The method of any one of claims 33 through 43, wherein the patient has fusion protein expression in one or in more than one brain region of the patient 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 or combinations thereof.

45. The method of any one of claims 33 through 44, wherein the patient has fusion protein expression in one or in more than one brain region of the patient wherein the brain region is selected from the group comprising, consisting, or consisting essentially of the brainstem, spinal cord, motor cortex, temporal cortex, thalamus or combinations thereof.

46. The method of any one of claims 33 through 45, wherein the patient has fusion protein expression in the pons region of the brainstem, cervical level of the spinal cord, precentral gyrus region of the motor cortex, temporal cortex, and thalamus.

47. The method of any one of claims 33 through 46, wherein the fusion protein is not expressed in S100β-positive glial cells.

48. The method of any one of claims 33 through 47, wherein the fusion protein is expressed in ChAT-positive motor neurons in the spinal cord.

49. The method of any one of claims 33 through 48, wherein the fusion protein expression is restricted to neurons by a hSYN1 promotor.

50. The method of any one of claims 33 through 49, wherein the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition.

51. The method of any one of claims 33 through 50, wherein the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition in more than one brain cell of the subject wherein the brain cell is 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.

52. The method of any one of claims 33 through 51, wherein the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition in more than one brain region of the patient 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 or combinations thereof.

53. The method of any one of claims 33 through 52, wherein the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition in more than one brain region of the patient wherein the brain region is selected from the group comprising, consisting, or consisting essentially of the brainstem, spinal cord, motor cortex, temporal cortex, thalamus or combinations thereof.

54. The method of any one of claims 33 through 53, wherein the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition in the pons region of the brainstem, cervical level of the spinal cord, precentral gyrus region of the motor cortex, temporal cortex, and thalamus.

55. The method of any one of claims 33 through 54, wherein the patient does not show tau repression compared to prior to introducing of the composition or pharmaceutical composition in S100β-positive glial cells.

56. The method of any one of claims 33 through 55, wherein the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition in ChAT-positive motor neurons in the spinal cord.

57. The method of any one of claims 33 through 56, wherein the patient has tau repression compared to prior to introducing of the composition or pharmaceutical composition in a plurality of NeuN-positive cells wherein fusion protein expression is below the limit of detection of an In Situ Hybridization assay

58. The method of any one of claims 33 through 57, wherein the patient is a human.

59. Use of a composition of any one of claims 1-21 for the manufacture of a medicament for use in the method of any one of claims 33-58.