

identical to an amino acid sequence provided in **Tables 2-3** or **FIG. 33** inserted at an amino acid position 588_589 within SEQ ID NO: 1, wherein the AAV capsid protein is characterized by at least one of an increased specificity and an increased transduction efficiency when measured in a central nervous system (CNS) in a subject when delivered to the subject systemically, relative to a native AAV capsid protein provided in SEQ ID NO: 1. In some examples, the second amino acid sequence is at least 71.4% identical to the amino acid sequence provided in **Tables 2-3** or **FIG. 33**. In some examples, the second amino acid sequence is at least 86.7% identical to the amino acid sequence provided in **Tables 2-3** or **FIG. 33**. In some examples, the second amino acid sequence is selected from the group consisting of TALKPFL, TTLKPFL, TLQIPFK, TMQKPFI, SIERPFK, RYQGDSV, and TTLKPFS. In some examples, the AAV capsid protein is present in VP1, VP2, and VP3 of the AAV capsid. In some examples, the AAV capsid is chimeric. In some examples, 60 copies of the AAV capsid protein are assembled into the AAV capsid. In some examples, the CNS comprises a cell-type selected from the group consisting of a neuron, an oligodendrocyte, an astrocyte, and a brain vascular cell. In some examples, the CNS comprises a tissue that is selected from the group consisting of a brain, a thalamus, a cortex, a striatum, a ventral midbrain, and a spinal cord. In some examples, the AAV capsid protein further comprises an amino acid substitution A587D. In some examples, the AAV capsid protein further comprises an amino acid substitution Q588G. In some examples, the AAV capsid protein further comprises an amino acid substitution comprising A589N. In some examples, the AAV capsid protein further comprises an amino acid substitution comprising Q590P. In some examples, the second amino acid sequence at the amino acid position 588_589 within SEQ ID NO: 1 is not TLAVPFK, KFPVALT, SVSKPFL, FTLTPK, MNATKNV, NGGTSSS, TRTNPEA, or YTLSQGW. In some examples, the AAV capsid is isolated and purified. In some examples, the AAV capsid is formulated as a pharmaceutical formulation for intravenous administration to treat a disease or a condition of the liver, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier. In some examples, the pharmaceutical formulation further comprises a therapeutic agent. In another aspect, the present disclosure provides a use of the AAV capsid as described herein in the manufacture of a pharmaceutical formulation for treating a disease or a condition of the CNS, the pharmaceutical formulation

further comprising a pharmaceutically acceptable carrier, wherein the pharmaceutical formulation is to be intravenously administered.

[0009] In another aspect, the present disclosure provides an AAV capsid comprising an AAV capsid protein comprising a seven-amino-acid insertion (X1-X2-X3-X4-X5-X6-X7) between amino acid 588 and amino acid 589 in an amino acid sequence of the AAV capsid protein provided in SEQ ID NO: 1, wherein: X1 is an amino acid selected from the group consisting of E, D, G, R, S and T; X2 is an amino acid selected from the group consisting of A, G, I, L, M, N, Q, T, and Y; X3 is an amino acid selected from the group consisting of E, K, L, T, and Q; and X4 is an amino acid selected from the group consisting of G, I, K, L, R, T, and V. Examples disclosed herein provide **AAV capsids** comprising an AAV capsid protein comprising a seven amino acid insertion (X1-X2-X3-X4-X5-X6-X7) between amino acid 588 and amino acid 589 in an amino acid sequence of the AAV capsid protein provided in SEQ ID NO: 1, wherein X1 is an amino acid selected from the group consisting of E, D, G, R, S and T. In some examples, X2 is an amino acid selected from the group consisting of A, G, I, L, M, N, Q, T, and Y. In some examples, X3 is an amino acid selected from the group consisting of E, K, L, T, and Q. In some examples, X4 is an amino acid selected from the group consisting of G, I, K, L, R, T, and V. In some examples, X5 is an amino acid selected from the group consisting of A, D, G, P, L, Q, and V. In some examples, X6 is an amino acid selected from the group consisting of F, K, N, P, Q, S, and V. In some examples, X7 is an amino acid selected from the group consisting of I, K, L, P, and V. In some examples, the seven amino acid insertion is selected from the group consisting of TALKPFL, TTLKPFL, TLQIPFK, TMQKPFI, SIERPFK, RYQGDSV, and TTLKPFS. In some examples, the AAV capsid protein is present in VP1, VP2, and VP3 of the AAV capsid. In some examples, the AAV capsid is chimeric. In some examples, 60 copies of the AAV capsid protein are assembled into the AAV capsid. In some examples, the AAV capsid protein is characterized by at least one of an increased specificity and an increased transduction efficiency when measured in a central nervous system (CNS) in a subject when delivered to the subject systemically, relative to a native AAV capsid protein provided in SEQ ID NO: 1. In some examples, the CNS comprises a cell-type selected from the group consisting of a neuron, a glial cell, an oligodendrocyte, an ependymal cell, an astrocyte, a Schwann cell, a satellite cell, and an enteric glial cell. In some examples, the CNS comprises a tissue that is selected from the group

consisting of a brain, a thalamus, a cortex, a striatum, a ventral midbrain, and a spinal cord. In some examples, the AAV capsid protein further comprises an amino acid substitution comprising A587D. In some examples, the AAV capsid protein further comprises an amino acid substitution comprising Q588G. In some examples, the AAV capsid protein further comprises an amino acid substitution comprising A589N. In some examples, the AAV capsid protein further comprises an amino acid substitution comprising Q590P. In some examples, the seven amino acid insertion is not TLAVPFK, KFPVALT, SVSKPFL, FTLTTPK, MNATKNV, NGGTSSS, TRTNPEA, or YTLSQGW. In some examples, the AAV capsid is isolated and purified. In some examples, the AAV capsid is formulated as a pharmaceutical formulation for intravenous administration to treat a disease or a condition of the liver, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier. In some examples, the pharmaceutical formulation further comprises a therapeutic agent.

[0010] Aspects provided herein provide AAV **capsids** comprising: (a) an AAV capsid protein comprising: (i) a first amino acid sequence that is at least 98% identical to amino acid 217 to amino acid 736 of SEQ ID NO: 1; and (ii) a second amino acid sequence at least 57.1% identical to an amino acid sequence provided in **Table 4** or **FIG. 35** at an amino acid position 588_589 within SEQ ID NO: 1, wherein the AAV capsid protein is characterized by at least one of an increased specificity and an increased transduction efficiency when measured in a liver in a subject when delivered to the subject systemically, relative to a native AAV capsid protein provided in SEQ ID NO: 1. In some examples, the second amino acid sequence is at least 71.4% identical to the amino acid sequence provided in **Table 4** or **FIG. 35**. In some examples, the second amino acid sequence is at least 86.7% identical to the amino acid sequence provided in **Table 4** or **FIG. 35**. In some examples, the second amino acid sequence is selected from the group consisting of KAYSVQV, PSGSARS, and RTANALG. In some examples, the AAV capsid protein is present in VP1, VP2, and VP3 of the AAV capsid. In some examples, the AAV capsid is chimeric. In some examples, 60 copies of the AAV capsid protein are assembled into the AAV capsid. In some examples, the AAV capsid is isolated and purified. In some examples, the AAV capsid is formulated as a pharmaceutical formulation for intravenous administration to treat a disease or a condition of the liver, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier. In some examples, the pharmaceutical formulation further

comprises a therapeutic agent. In another aspect, the present disclosure provides a use of the AAV capsid as described herein in the manufacture of a pharmaceutical formulation for treating a disease or a condition of the liver, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier, wherein the pharmaceutical formulation is to be intravenously administered.

[0011] Examples disclosed herein provide **AAV capsid proteins** comprising: (i) a first amino acid sequence that is at least 98% identical to amino acid 217 to amino acid 736 of SEQ ID NO: 1; and (ii) a second amino acid sequence at least 57.1% identical to an amino acid sequence provided in **Tables 2-3** or **FIG. 33** inserted at an amino acid position 588_589 within SEQ ID NO: 1, wherein the AAV capsid protein is characterized by at least one of an increased specificity and an increased transduction efficiency when measured in a central nervous system (CNS) in a subject when delivered to the subject systemically, relative to a native AAV capsid protein provided in SEQ ID NO: 1. In some examples, the second amino acid sequence is at least 71.4% identical to the amino acid sequence provided in **Tables 2-3** or **FIG. 33**. In some examples, the second amino acid sequence is at least 86.7% identical to the amino acid sequence provided in **Tables 2-3** or **FIG. 33**. In some examples, the second amino acid sequence is selected from the group consisting of TALKPFL, TTLKPFL, TLQIPFK, TMQKPFI, SIERPFK, RYQGDSV, and TTLKPFS. In some examples, the AAV capsid protein is present in VP1, VP2, and VP3 of an AAV capsid. In some examples, the AAV capsid is chimeric. In some examples, 60 copies of the AAV capsid protein are assembled into the AAV capsid. In some examples, the CNS comprises a cell-type selected from the group consisting of a neuron, an oligodendrocyte, an astrocyte, and a brain vascular cell. In some examples, the CNS comprises a tissue that is selected from the group consisting of a brain, a thalamus, a cortex, a striatum, a ventral midbrain, and a spinal cord. In some examples, the AAV capsid protein further comprises an amino acid substitution A587D. In some examples, the AAV capsid protein further comprises an amino acid substitution Q588G. In some examples, the AAV capsid protein further comprises an amino acid substitution comprising A589N. In some examples, the AAV capsid protein further comprises an amino acid substitution comprising Q590P. In some examples, the second amino acid sequence at the amino acid position 588_589 within SEQ ID NO: 1 is not TLAVPFL, KFPVALT, SVSKPFL, FTLTPFK, MNATKNV, NGGTSSS, TRTNPEA, or YTLSQGW. In some

examples, the AAV capsid protein is isolated and purified. In some examples, the AAV capsid protein is formulated as a pharmaceutical formulation for intravenous administration to treat a disease or a condition of the liver, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier. In some examples, the pharmaceutical formulation further comprises a therapeutic agent.

[0012] Examples disclosed herein provide **AAV capsids proteins** comprising a seven amino acid insertion (X1-X2-X3-X4-X5-X6-X7) between amino acid 588 and amino acid 589 in an amino acid sequence of the AAV capsid protein provided in SEQ ID NO: 1, wherein X1 is an amino acid selected from the group consisting of E, D, G, R, S and T. In some examples, X2 is an amino acid selected from the group consisting of A, G, I, L, M, N, Q, T, and Y. In some examples, X3 is an amino acid selected from the group consisting of E, K, L, T, and Q. In some examples, X4 is an amino acid selected from the group consisting of G, I, K, L, R, T, and V. In some examples, X5 is an amino acid selected from the group consisting of A, D, G, P, L, Q, and V. In some examples, X6 is an amino acid selected from the group consisting of F, K, N, P, Q, S, and V. In some examples, X7 is an amino acid selected from the group consisting of I, K, L, P, and V. In some examples, the seven amino acid insertion is selected from the group consisting of TALKPFL, TTLKPFL, TLQIPFK, TMQKPFI, SIERPFK, RYQGDSV, and TTLKPFS. In some examples, the AAV capsid protein is present in VP1, VP2, and VP3 of a AAV capsid. In some examples, the AAV capsid is chimeric. In some examples, 60 copies of the AAV capsid protein are assembled into the AAV capsid. In some examples, the AAV capsid protein is characterized by at least one of an increased specificity and an increased transduction efficiency when measured in a central nervous system (CNS) in a subject when delivered to the subject systemically, relative to a native AAV capsid protein provided in SEQ ID NO: 1. In some examples, the CNS comprises a cell-type selected from the group consisting of a neuron, a glial cell, a oligodendrocyte, an ependymal cell, an astrocyte, a Schwann cell, a satellite cell, and an enteric glial cell. In some examples, the CNS comprises a tissue that is selected from the group consisting of a brain, a thalamus, a cortex, a striatum, a ventral midbrain, and a spinal cord. In some examples, the AAV capsid protein further comprises an amino acid substitution comprising A587D. In some examples, the AAV capsid protein further comprises an amino acid substitution comprising Q588G. In some examples, the AAV capsid protein further comprises an amino acid

substitution comprising A589N. In some examples, the AAV capsid protein further comprises an amino acid substitution comprising Q590P. In some examples, the seven amino acid insertion is not TLAVPFK, KFPVALT, SVSKPFL, FTLTTPK, MNATKNV, NGGTSSS, TRTNPEA, or YTLSQGW. In some examples, the AAV capsid protein is isolated and purified. In some examples, the AAV capsid protein is formulated as a pharmaceutical formulation for intravenous administration to treat a disease or a condition of the liver, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier. In some examples, the pharmaceutical formulation further comprises a therapeutic agent.

[0013] Examples provided herein provide **AAV capsids proteins** comprising: (i) a first amino acid sequence that is at least 98% identical to amino acid 217 to amino acid 736 of SEQ ID NO: 1; and (ii) a second amino acid sequence at least 57.1% identical to an amino acid sequence provided in **Table 4** or **FIG. 35** at an amino acid position 588_589 within SEQ ID NO: 1, wherein the AAV capsid protein is characterized by at least one of an increased specificity and an increased transduction efficiency when measured in a liver in a subject when delivered to the subject systemically, relative to a native AAV capsid protein provided in SEQ ID NO: 1. In some examples, the second amino acid sequence is at least 71.4% identical to the amino acid sequence provided in **Table 4** or **FIG. 35**. In some examples, the second amino acid sequence is at least 86.7% identical to the amino acid sequence provided in **Table 4** or **FIG. 35**. In some examples, the second amino acid sequence is selected from the group consisting of KAYSVQV, PSGSARS, and RTANALG. In some examples, the AAV capsid protein is present in VP1, VP2, and VP3 of an AAV capsid. In some examples, the AAV capsid is chimeric. In some examples, 60 copies of the AAV capsid protein are assembled into the AAV capsid. In some examples, the AAV capsid protein is isolated and purified. In some examples, the AAV capsid protein is formulated as a pharmaceutical formulation for intravenous administration to treat a disease or a condition of the liver, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier. In some examples, the pharmaceutical formulation further comprises a therapeutic agent.

[0014] Examples disclosed herein comprise **plasmid vectors** comprising a nucleic acid sequence encoding the AAV capsids and AAV capsid proteins described herein. In some instances, the plasmid vector is bacterial. In some instances, the plasmid vector is derived from

Escherichia coli. In some instances, the nucleic acid sequence comprises, in a 5' to 3' direction: (1) a 5' inverted terminal repeat (ITR) sequence, (2) a Replication (*Rep*) gene, (3) a Capsid (*Cap*) gene, and (4) a 3' ITR, wherein the *Cap* gene encodes the AAV capsid protein described herein. In some instances, the plasmid vector encodes a pseudotyped AAV capsid protein. In some instances, the *Cap* gene is derived from the deoxyribose nucleic acid (DNA) provided in any one of SEQ ID NOs: 6-10. In some instances, the nucleic acid sequence comprising the *Cap* gene is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of the DNA sequences provided in U.S. App. Ser. No. 16/582,635, incorporated herein by reference. In some instances, the 5' ITR and the 3' ITR are derived from an AAV2 serotype. In some instances, the 5' ITR and the 3' ITR are derived from an AAV5 serotype. In some instances, the 5' ITR and the 3' ITR are derived from an AAV9 serotype.

[0015] Examples disclosed herein provide methods of treating a disease or condition in a subject comprising administering a therapeutically effective amount of a pharmaceutical formulation comprising the AAV capsid protein or the AAV capsid of the present disclosure. In some examples, the disease or the condition is a disease or a condition of a central nervous system (CNS) or a liver of the subject. In some examples, the disease or condition of the liver is selected from the group consisting of Alagille Syndrome, Alcohol-Related Liver Disease, Alpha-1 Antitrypsin Deficiency, Autoimmune Hepatitis, Benign Liver Tumors, Biliary Atresia, Cirrhosis, Crigler-Najjar Syndrome, Galactosemia, Gilbert Syndrome, Hemochromatosis, Hepatic Encephalopathy, Hepatitis A, Hepatitis B, Hepatitis C, Hepatorenal Syndrome, Intrahepatic Cholestasis of Pregnancy (ICP), Lysosomal Acid Lipase Deficiency (LAL-D), Liver Cysts, Liver Cancer, Newborn Jaundice, Non-Alcoholic Fatty Liver Disease, Primary Biliary Cholangitis (PBC), Primary Sclerosing Cholangitis (PSC), Reye Syndrome, Type I Glycogen Storage Disease, and Wilson Disease. In some examples, the disease or condition of the CNS is selected from group consisting of Absence of the Septum Pellucidum, Acid Lipase Disease, Acid Maltase Deficiency, Acquired Epileptiform Aphasia, Acute Disseminated Encephalomyelitis, Attention Deficit-Hyperactivity Disorder (ADHD), Adie's Pupil, Adie's Syndrome, Adrenoleukodystrophy, Agenesis of the Corpus Callosum, Agnosia, Aicardi Syndrome, Aicardi-Goutieres Syndrome Disorder, AIDS -Neurological Complications, Alexander Disease, Alpers' Disease, Alternating Hemiplegia, Alzheimer's Disease, Amyotrophic Lateral Sclerosis (ALS),

Anencephaly, Aneurysm, Angelman Syndrome, Angiomatosis, Anoxia, Antiphospholipid Syndrome, Aphasia, Apraxia, Arachnoid Cysts, Arachnoiditis, Arnold-Chiari Malformation, Arteriovenous Malformation, Asperger Syndrome, Ataxia, Ataxia Telangiectasia, Ataxias and Cerebellar or Spinocerebellar Degeneration, Atrial Fibrillation and Stroke, Attention Deficit-Hyperactivity Disorder, Autism Spectrum Disorder, Autonomic Dysfunction, Back Pain, Barth Syndrome, Batten Disease, Becker's Myotonia, Behcet's Disease, Bell's Palsy, Benign Essential Blepharospasm, Benign Focal Amyotrophy, Benign Intracranial Hypertension, Bernhard-Roth Syndrome, Binswanger's Disease, Blepharospasm, Bloch-Sulzberger Syndrome, Brachial Plexus Birth Injuries, Brachial Plexus Injuries, Bradbury-Eggleston Syndrome, Brain and Spinal Tumors, Brain Aneurysm, Brain Injury, Brown-Sequard Syndrome, Bulbospinal Muscular Atrophy, Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), Canavan Disease, Carpal Tunnel Syndrome, Causalgia, Cavernomas, Cavernous Angioma, Cavernous Malformation, Central Cervical Cord Syndrome, Central Cord Syndrome, Central Pain Syndrome, Central Pontine Myelinolysis, Cephalic Disorders, Ceramidase Deficiency, Cerebellar Degeneration, Cerebellar Hypoplasia, Cerebral Aneurysms, Cerebral Arteriosclerosis, Cerebral Atrophy, Cerebral Beriberi, Cerebral Cavemous Malformation, Cerebral Gigantism, Cerebral Hypoxia, Cerebral Palsy, Cerebro-Oculo-Facio-Skeletal Syndrome (COFS), Charcot-Marie-Tooth Disease, Charcot-Marie-Tooth syndrome, classical rhizomelic chondrodysplasia punctata (RCDP), Chiari Malformation, Cholesterol Ester Storage Disease, Chorea, Choreoacanthocytosis, Chronic Inflammatory Demyelinating Polyneuropathy (CIDP), Chronic Orthostatic Intolerance, Chronic Pain, Cockayne Syndrome Type II, Coffin Lowry Syndrome, Colpocephaly, Coma, Complex Regional Pain Syndrome, Congenital Facial Diplegia, Congenital Myasthenia, Congenital Myopathy, Congenital Vascular Cavernous Malformations, Corticobasal Degeneration, Cranial Arteritis, Craniosynostosis, Cree encephalitis, Creutzfeldt- Jakob Disease, Cumulative Trauma Disorders, Cushing's Syndrome, Cytomegalic Inclusion Body Disease, Cytomegalovirus Infection, Dancing Eyes-Dancing Feet Syndrome, Dandy-Walker Syndrome, Dawson Disease, Deafness, De Morsier's Syndrome, Dejerine-Klumpke Palsy, Dementia, Dementia -Multi -Infarct, Dementia - Semantic, Dementia - Subcortical, Dementia With Lewy Bodies, Dentate Cerebellar Ataxia, Dentatorubral Atrophy, Dermatomyositis, Developmental Dyspraxia, Devic's Syndrome, Diabetic Neuropathy, Diffuse

Sclerosis, Dravet Syndrome, Duchenne muscular dystrophy, Dysautonomia, Dysgraphia, Dyslexia, Dysphagia, Dyspraxia, Dyssynergia Cerebellaris Myoclonica, Dyssynergia Cerebellaris Progressiva, Dystonias, Early Infantile Epileptic Encephalopathy, Empty Sella Syndrome, Encephalitis, Encephalitis Lethargica, Encephaloceles, Encephalopathy, Encephalopathy (familial infantile), Encephalotrigeminal Angiomatosis, Epilepsy, Epileptic Hemiplegia, Erb's Palsy, Erb-Duchenne and Dejerine-Klumpke Palsies, Essential Tremor, Extrapontine Myelinolysis, Fabry Disease, Fahr's Syndrome, Fainting, Familial Dysautonomia, Familial Hemangioma, Familial Idiopathic Basal Ganglia Calcification, Familial Periodic Paralysis, Familial Spastic Paralysis, Farber's Disease, Febrile Seizures, Fibromuscular Dysplasia, Fisher Syndrome, Floppy Infant Syndrome, Foot Drop, Friedreich's Ataxia, Frontotemporal Dementia, Gaucher Disease, Generalized Gangliosidoses, Gerstmann's Syndrome, Gerstmann-Straussler-Scheinker Disease, Giant Axonal Neuropathy, Giant Cell Arteritis, Giant Cell Inclusion Disease, glioblastoma, Globoid Cell Leukodystrophy, Glossopharyngeal Neuralgia, Glycogen Storage Disease, Guillain-Barre Syndrome, Hallervorden-Spatz Disease, Head Injury, Headache, Hemicrania Continua, Hemifacial Spasm, Hemiplegia Alterans, Hereditary Neuropathies, Hereditary Spastic Paraplegia, Heredopathia Atactica Polyneuritiformis, Herpes Zoster, Herpes Zoster Oticus, Hirayama Syndrome, Holmes-Adie syndrome, Holoprosencephaly, HTLV-1 Associated Myelopathy, Hughes Syndrome, Huntington's Disease, Hydranencephaly, Hydrocephalus, Hydrocephalus - Normal Pressure, Hydromyelia, Hypercortisolism, Hypersomnia, Hypertonia, Hypotonia, Hypoxia, Immune-Mediated Encephalomyelitis, Inclusion Body Myositis, Incontinentia Pigmenti, Infantile Hypotonia, Infantile Neuroaxonal Dystrophy, Infantile Phytanic Acid Storage Disease, Infantile Refsum Disease, Infantile Spasms, Inflammatory Myopathies, Iniencephaly, Intestinal Lipodystrophy, Intracranial Cysts, Intracranial Hypertension, Isaacs' Syndrome, Joubert Syndrome, Kearns-Sayre Syndrome, Kennedy's Disease, Kinsbourne syndrome, Kleine-Levin Syndrome, Klippel-Feil Syndrome, Klippel-Trenaunay Syndrome (KTS), Kliiver-Bucy Syndrome, Korsakoff's Amnesic Syndrome, Krabbe Disease, Kugelberg-Welander Disease, Kuru, Lambert-Eaton Myasthenic Syndrome, Landau-Kleffner Syndrome, Lateral Femoral Cutaneous Nerve Entrapment, Lateral Medullary Syndrome, Learning Disabilities, Leigh's Disease, Lennox-Gastaut Syndrome, Lesch-Nyhan Syndrome, Leukodystrophy, Levine-

Critchley Syndrome, Lewy Body Dementia, Lipid Storage Diseases, Lipoid Proteinosis, Lissencephaly, Locked-In Syndrome, Lou Gehrig's Disease, Lupus -Neurological Sequelae, Lyme Disease - Neurological Complications, Machado- Joseph Disease, Macrencephaly, Megalencephaly, Melkersson-Rosenthal Syndrome, Meningitis, Meningitis and Encephalitis, Menkes Disease, Meralgia Paresthetica, Metachromatic Leukodystrophy, Microcephaly, Migraine, Miller Fisher Syndrome, Mini Stroke, Mitochondrial Myopathy, Moebius Syndrome, Monomelic Amyotrophy, Motor Neuron Diseases, Moyamoya Disease, Mucopolidoses, Mucopolysaccharidoses, Multi-Infarct Dementia, Multifocal Motor Neuropathy, Multiple Sclerosis, Multiple System Atrophy, Multiple System Atrophy with Orthostatic Hypotension, Muscular Dystrophy, Myasthenia -Congenital, Myasthenia Gravis, Myelinoclastic Diffuse Sclerosis, Myoclonic Encephalopathy of Infants, Myoclonus, Myopathy, Myopathy- Congenital, Myopathy -Thyrotoxic, Myotonia, Myotonia Congenita, Narcolepsy, Neuroacanthocytosis, Neurodegeneration with Brain Iron Accumulation, Neurofibromatosis, Neuroleptic Malignant Syndrome, Neurological Complications of AIDS, Neurological Complications of Lyme Disease, Neurological Consequences of Cytomegalovirus Infection, Neurological Manifestations of Pompe Disease, Neurological Sequelae Of Lupus, Neuromyelitis Optica, Neuromyotonia, Neuronal Ceroid Lipofuscinosis, Neuronal Migration Disorders, Neuropathy- Hereditary, Neurosarcoidosis, Neurosyphilis, Neurotoxicity, Nevus Cavernosus, Niemann-Pick Disease, O'Sullivan-McLeod Syndrome, Occipital Neuralgia, Ohtahara Syndrome, Olivopontocerebellar Atrophy, Opsoclonus Myoclonus, Orthostatic Hypotension, Overuse Syndrome, Pain -Chronic, Pantothenate Kinase-Associated Neurodegeneration, Paraneoplastic Syndromes, Paresthesia, Parkinson's Disease, Paroxysmal Choreoathetosis, Paroxysmal Hemicrania, Parry -Romberg, Pelizaeus-Merzbacher Disease, Pena Shokeir II Syndrome, Perineural Cysts, Periodic Paralysis, Peripheral Neuropathy, Periventricular Leukomalacia, Persistent Vegetative State, Pervasive Developmental Disorders, Phytanic Acid Storage Disease, Pick's Disease, Pinched Nerve, Piriformis Syndrome, Pituitary Tumors, Polymyositis, Pompe Disease, Porencephaly, Post-Polio Syndrome, Postherpetic Neuralgia, Postinfectious Encephalomyelitis, Postural Hypotension, Postural Orthostatic Tachycardia Syndrome, Postural Tachycardia Syndrome, Primary Dentatum Atrophy, Primary Lateral Sclerosis, Primary Progressive Aphasia, Prion Diseases, Progressive Hemifacial Atrophy, Progressive Locomotor Ataxia, Progressive Multifocal

Leukoencephalopathy, Progressive Sclerosing Poliodystrophy, Progressive Supranuclear Palsy, Prosopagnosia, Pseudo-Torch syndrome, Pseudotoxoplasmosis syndrome, Pseudotumor Cerebri, Psychogenic Movement, Ramsay Hunt Syndrome I, Ramsay Hunt Syndrome II, Rasmussen's Encephalitis, Reflex Sympathetic Dystrophy Syndrome, Refsum Disease, Refsum Disease - Infantile, Repetitive Motion Disorders, Repetitive Stress Injuries, Restless Legs Syndrome, Retrovirus-Associated Myelopathy, Rett Syndrome, Reye's Syndrome, Rheumatic Encephalitis, Riley-Day Syndrome, Sacral Nerve Root Cysts, Saint Vitus Dance, Salivary Gland Disease, Sandhoff Disease, Schilder's Disease, Schizencephaly, Seitelberger Disease, Seizure Disorder, Semantic Dementia, Septo-Optic Dysplasia, Severe Myoclonic Epilepsy of Infancy (SMEI), Shaken Baby Syndrome, Shingles, Shy-Drager Syndrome, Sjogren's Syndrome, Sleep Apnea, Sleeping Sickness, Sotos Syndrome, Spasticity, Spina Bifida, Spinal Cord Infarction, Spinal Cord Injury, Spinal Cord Tumors, Spinal Muscular Atrophy, Spinocerebellar Atrophy, Spinocerebellar Degeneration, Steele-Richardson-Olszewski Syndrome, Stiff-Person Syndrome, Striatonigral Degeneration, Stroke, Sturge-Weber Syndrome, Subacute Sclerosing Panencephalitis, Subcortical Arteriosclerotic Encephalopathy, Short-lasting, Unilateral, Neuralgiform (SUNCT) Headache, Swallowing Disorders, Sydenham Chorea, Syncope, Syphilitic Spinal Sclerosis, Syringohydromyelia, Syringomyelia, Systemic Lupus Erythematosus, Tabes Dorsalis, Tardive Dyskinesia, Tarlov Cysts, Tay-Sachs Disease, Temporal Arteritis, Tethered Spinal Cord Syndrome, Thomsen's Myotonia, Thoracic Outlet Syndrome, Thyrotoxic Myopathy, Tic Douloureux, Todd's Paralysis, Tourette Syndrome, Transient Ischemic Attack, Transmissible Spongiform Encephalopathies, Transverse Myelitis, Traumatic Brain Injury, Tremor, Trigeminal Neuralgia, Tropical Spastic Paraparesis, Troyer Syndrome, Tuberous Sclerosis, Vascular Erectile Tumor, Vasculitis Syndromes of the Central and Peripheral Nervous Systems, Von Economo's Disease, Von Hippel-Lindau Disease (VHL), Von Recklinghausen's Disease, Wallenberg's Syndrome, Werdnig-Hoffman Disease, Wernicke-Korsakoff Syndrome, West Syndrome, Whiplash, Whipple's Disease, Williams Syndrome, Wilson Disease, Wolman's Disease, and X-Linked Spinal and Bulbar Muscular Atrophy. In some examples, the pharmaceutical formulation comprises a therapeutic nucleic acid encoding a therapeutic gene expression product. In some instances, the therapeutic gene expression product is effective to modulate an activity or an expression of a target gene or gene expression product

selected from the group consisting of Sarcoglycan Alpha (SGCA), glutamic acid decarboxylase 65 (GAD65), glutamic acid decarboxylase 67 (GAD67), CLN2, Nerve Growth Factor (NGF), Survival Of Motor Neuron 1, Telomeric (SMN1), Factor X (FIX), Retinoid Isomerohydrolase (RPE65), sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA2a), β -Glucocerebrosidase (GCase), Frataxin (FXN), Huntingtin (HTN), methyl-CpG binding protein 2 (MECP2), a peroxisomal biogenesis factor (PEX), progranulin (GRN), an antitubulin agent, copper-zinc superoxide dismutase (SOD1), Glucosylceramidase Beta (GBA), NPC Intracellular Cholesterol Transporter 1 (NPC1), and a NLRP3 inflammasome. In some instances, the therapeutic gene expression product comprises gene editing components. In some instances, the gene editing components are selected from the group consisting of small interfering RNA (siRNA), short hairpin RNA (shRNA), a microRNA (miRNA), artificial site-specific RNA endonuclease (ASRE), zinc finger endonuclease (ZFN), CRISPR/Cas, and transcription factor like effector nuclease (TALEN).

[0016] Aspects disclosed herein provide **methods of manufacturing** a recombinant AAV particle from the AAV capsid of the present disclosure, the method comprising: (a) introducing into a cell a nucleic acid comprising: (i) a first nucleic acid sequence encoding a therapeutic gene expression product; (ii) a second nucleic acid sequence encoding a recombinant viral genome comprising a capsid (*Cap*) gene modified to express the AAV capsid of the present disclosure; and (iii) a third nucleic acid sequence encoding an AAV helper virus genome; and (b) assembling the recombinant AAV particle, the recombinant AAV particle comprising the AAV capsid encapsidating the first nucleic acid.

[0017] Examples disclosed herein provide **methods of manufacturing** comprising: (a) introducing into a cell a nucleic acid comprising: (i) a first nucleic acid sequence encoding a therapeutic gene expression product enclosed by a 5' and a 3' inverted terminal repeat (ITR) sequence; (ii) a second nucleic acid sequence encoding a viral genome comprising a 5' ITR sequence, a Replication (*Rep*) gene, Capsid (*Cap*) gene, and a 3' ITR, wherein the *Cap* gene encodes the AAV capsid protein described herein; and (iii) a third nucleic acid sequence encoding a first helper virus protein selected from the group consisting of E4orf6, E2a, and VA RNA, and optionally, a second helper virus protein comprising E1a or E1b55k; (b) expressing in the cell the AAV capsid protein described herein; (c) assembling an AAV particle comprising

the AAV capsid proteins disclosed herein; and (d) packaging the first nucleic acid sequence in the AAV particle. In some instances, the cell is mammalian. In some instances, the cell is immortalized. In some instances, the immortalized cell is an embryonic stem cell. In some instances, the embryonic stem cell is a human embryonic stem cell. In some instances, the human embryonic stem cell is a human embryonic kidney 293 (HEK-293) cell. In some instances, the *Cap* gene is derived from the deoxyribose nucleic acid (DNA) provided in any one of SEQ ID NOs: 6-10. In some instances, the nucleic acid sequence comprising the *Cap* gene is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of the DNA sequences provided in U.S. App. Ser. No. 16/582,635, incorporated herein by reference. In some instances, the 5' ITR and the 3' ITR are derived from an AAV2 serotype. In some instances, the 5' ITR and the 3' ITR are derived from an AAV5 serotype. In some instances, the 5' ITR and the 3' ITR are derived from an AAV9 serotype. In some instances, the first nucleic acid sequence and the second nucleic acid sequence are in *trans*. In some instances, the first nucleic acid sequence and the second nucleic acid sequence are in *cis*. In some instances, the first nucleic acid sequence, the second nucleic acid sequence and the third nucleic acid sequence, are in *trans*.

[0018] Aspects disclosed herein provide **methods of manufacturing** a recombinant AAV particle, the method comprising: (a) providing a recombinant AAV genome comprising: (i) an AAV capsid gene, and (ii) a recognition sequence for a Cre recombinase, wherein the recognition sequence facilitates a recombinase-dependent change that is detectable, and wherein the recombinase recognition sequence comprises two Cre-recognition sites; (iii) transfecting a population of cells expressing the Cre recombinase with the recombinant AAV genome, whereby the Cre recombinase induces a recombination event to generate the recombinase-dependent change in the recombinant AAV genome, and wherein the recombinase-dependent change comprises an inversion of the sequence that is flanked by the Cre-recognition sites; (iv) detecting an increased rate of the recombinase-dependent change a target cell in the population of cells; (v) detecting a decreased rate of the recombinase-dependent change in an off-target cell in the population of cells; and (vi) identifying a recombinant AAV genome generated by the recombinase-dependent change, wherein said identified rAAV genome comprises the inversion, and wherein said identified recombinant AAV genome encodes an AAV capsid particle

characterized having an increased specificity for the target cell and a decreased specificity for the off-target cell. In some examples, the off-target cell is a hepatocyte. In some examples, the target cell is a cell selected from the group consisting of a neuron, a glial cell, an oligodendrocyte, an ependymal cell, an astrocyte, a Schwann cell, a satellite cell, and an enteric glial cell.

[0019] In a further aspect, the present disclosure provides a recombinant vector comprising a nucleic acid encoding the AAV capsid protein of any one as described herein. Aspects disclosed herein provide **kits** comprising: (a) a first vector comprising the recombinant vector of the present disclosure; (b) a second vector encoding a helper virus protein; and (c) a third vector comprising a therapeutic nucleic acid encoding a therapeutic gene expression product.

[0019a] In a further aspect, the present disclosure provides the AAV capsid protein as described herein for use in therapy. In a further aspect, the present disclosure provides a use of the AAV capsid protein as described herein in the manufacture of a medicament for treating a disease or a condition of a central nervous system or a liver of the subject.

[0020] Examples disclosed herein provide **kits** comprising: (a) a first vector comprising a first nucleic acid sequence encoding a viral genome comprising in a 5' to 3' direction: (i) a 5' inverted terminal repeat (ITR) sequence; (ii) a Replication (*Rep*) gene; (iii) a Capsid (*Cap*) gene encoding the AAV capsid proteins described herein, and (iv) a 3' ITR; and (b) optionally, a second vector comprising a second nucleic acid sequence encoding a helper virus protein comprising at least one of E4orf6, E2a, VA RNA, E1a and E1b55k. In some instances, the kit further comprises a cell. In some instances, the cell is mammalian. In some instances, the cell is immortalized. In some instances, the immortalized cell is an embryonic stem cell. In some instances, the embryonic stem cell is a human embryonic stem cell. In some instances, the human embryonic stem cell is a human embryonic kidney 293 (HEK-293) cell. In some instances, the kit further comprises an AAV vector comprising a heterologous nucleic acid encoding a therapeutic gene expression product. In some instances, the AAV vector is an episome.

INCORPORATION BY REFERENCE

[0021] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0023] **FIG. 1** shows a multiplexed selection approach to identify capsids with specific and broad tropisms. Steps 1-6 describe the workflow in Round-1 (R1) selection, steps 7-9 describe Round-2 (R2) selection using synthetic pool method, steps 1a, 2a, and 6a-b show the incorporation of deep sequencing to recover capsids after R1 and R2 selection, and steps 10-11 describe positive and/or negative selection criteria followed by variant characterization.

CLAIMS

1. An AAV capsid comprising:
 - a) an AAV capsid protein comprising:
 - i. a first amino acid sequence that is at least 98% identical to amino acid 217 to amino acid 736 of SEQ ID NO: 1; and
 - ii. a second amino acid sequence at least 57.1% identical to an amino acid sequence provided in Tables 2-3 or FIG. 33 inserted at an amino acid position 588_589 within SEQ ID NO: 1, wherein the AAV capsid protein is characterized by at least one of an increased specificity and an increased transduction efficiency when measured in a central nervous system (CNS) in a subject when delivered to the subject systemically, relative to a native AAV capsid protein provided in SEQ ID NO: 1.
2. The AAV capsid of claim 1, wherein the second amino acid sequence is at least 71.4% identical to the amino acid sequence provided in Tables 2-3 or FIG. 33.
3. The AAV capsid of claim 1, wherein the second amino acid sequence is at least 86.7% identical to the amino acid sequence provided in Tables 2-3 or FIG. 33.
4. The AAV capsid of claim 1, wherein the second amino acid sequence is selected from the group consisting of TALKPFL, TTLKPFL, TLQIPFK, TMQKPFI, SIERPFK, RYQGDSV, and TTLKPFS.
5. The AAV capsid of claim 1, wherein the AAV capsid protein is present in VP1, VP2, and VP3 of the AAV capsid.
6. The AAV capsid of claim 1, wherein the AAV capsid is chimeric.
7. The AAV capsid of claim 1, wherein 60 copies of the AAV capsid protein are assembled into the AAV capsid.
8. The AAV capsid protein of claim 1, wherein the CNS comprises a cell-type selected from the group consisting of a neuron, an oligodendrocyte, an astrocyte, and a brain vascular cell.
9. The AAV capsid of claim 1, wherein the CNS comprises a tissue that is selected from the group consisting of a brain, a thalamus, a cortex, a striatum, a ventral midbrain, and a spinal cord.

10. The AAV capsid of claim 1, wherein the AAV capsid protein further comprises an amino acid substitution A587D or Q588G.
11. The AAV capsid protein of claim 1, wherein the AAV capsid protein further comprises an amino acid substitution A589N or Q590P.
12. The AAV capsid of claim 1, wherein the second amino acid sequence at the amino acid position 588_589 within SEQ ID NO: 1 is not TLAVPFK, KFPVALT, SVSKPFL, FTLTTPK, MNATKNV, NGGTSSS, TRTNPEA, or YTLSQGW.
13. The AAV capsid of claim 1 that is isolated and purified.
14. Use of the AAV capsid of claim 1 in the manufacture of a pharmaceutical formulation for treating a disease or a condition of the CNS, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier, wherein the pharmaceutical formulation is to be intravenously administered.
15. The use of claim 14, wherein the pharmaceutical formulation further comprises a therapeutic agent.
16. An AAV capsid comprising an AAV capsid protein comprising a seven-amino-acid insertion (X1-X2-X3-X4-X5-X6-X7) between amino acid 588 and amino acid 589 in an amino acid sequence of the AAV capsid protein provided in SEQ ID NO: 1, wherein:
 - X1 is an amino acid selected from the group consisting of E, D, G, R, S and T;
 - X2 is an amino acid selected from the group consisting of A, G, I, L, M, N, Q, T, and Y;
 - X3 is an amino acid selected from the group consisting of E, K, L, T, and Q; and
 - X4 is an amino acid selected from the group consisting of G, I, K, L, R, T, and V.
17. The AAV capsid protein of claim 16, wherein X5 is an amino acid selected from the group consisting of A, D, G, P, L, Q, and V.
18. The AAV capsid protein of claim 17, wherein X6 is an amino acid selected from the group consisting of F, K, N, P, Q, S, and V.

19. The AAV capsid protein of claim 18, wherein X7 is an amino acid selected from the group consisting of I, K, L, P, and V.
20. The AAV capsid of claim 16, wherein the seven-amino-acid insertion is selected from the group consisting of TALKPFL, TTLKPFL, TLQIPFK, TMQKPFI, SIERPFK, RYQGDSV, and TTLKPFS.
21. The AAV capsid of claim 16, wherein the AAV capsid protein is present in VP1, VP2, and VP3 of the AAV capsid.
22. The AAV capsid of claim 16, wherein the AAV capsid is chimeric.
23. The AAV capsid of claim 16, wherein 60 copies of the AAV capsid protein are assembled into the AAV capsid.
24. The AAV capsid of claim 16, wherein the AAV capsid protein is characterized by at least one of an increased specificity and an increased transduction efficiency when measured in a central nervous system (CNS) in a subject when delivered to the subject systemically, relative to a native AAV capsid protein provided in SEQ ID NO: 1
25. The AAV capsid protein of claim 24, wherein the CNS comprises a cell-type selected from the group consisting of a neuron, a glial cell, an oligodendrocyte, an ependymal cell, an astrocyte, a Schwann cell, a satellite cell, and an enteric glial cell.
26. The AAV capsid of claim 24, wherein the CNS comprises a tissue that is selected from the group consisting of a brain, a thalamus, a cortex, a striatum, a ventral midbrain, and a spinal cord.
27. The AAV capsid of claim 16, wherein the AAV capsid protein further comprises an amino acid substitution comprising A587D or Q588G.
28. The AAV capsid protein of claim 16, wherein the AAV capsid protein further comprises an amino acid substitution comprising A589N or Q590P.
29. The AAV capsid of claim 16, wherein the seven-amino-acid insertion is not TLAVPFK, KFPVALT, SVSKPFL, FTLTTPK, MNATKNV, NGGTSSS, TRTNPEA, or YTLSQGW.
30. The AAV capsid of claim 16 that is isolated and purified.
31. Use of the AAV capsid of claim 16 in the manufacture of a pharmaceutical formulation for treating a disease or a condition of the CNS, the pharmaceutical formulation further

comprising a pharmaceutically acceptable carrier, wherein the pharmaceutical formulation is to be intravenously administered.

32. The use of claim 31, wherein the pharmaceutical formulation further comprises a therapeutic agent.

33. An AAV capsid comprising:

a) an AAV capsid protein comprising:

- i. a first amino acid sequence that is at least 98% identical to amino acid 217 to amino acid 736 of SEQ ID NO: 1; and
- ii. a second amino acid sequence at least 57.1% identical to an amino acid sequence provided in Table 4 or FIG. 35 at an amino acid position 588_589 within SEQ ID NO: 1, wherein the AAV capsid protein is characterized by at least one of an increased specificity and an increased transduction efficiency when measured in a liver in a subject when delivered to the subject systemically, relative to a native AAV capsid protein provided in SEQ ID NO: 1.

34. The AAV capsid of claim 33, wherein the second amino acid sequence is at least 71.4% identical to the amino acid sequence provided in Table 4 or FIG. 35.

35. The AAV capsid of claim 33, wherein the second amino acid sequence is at least 86.7% identical to the amino acid sequence provided in Table 4 or FIG. 35.

36. The AAV capsid of claim 33, wherein the second amino acid sequence is selected from the group consisting of KAYSVQV, PSGSARS, and RTANALG.

37. The AAV capsid of claim 33, wherein the AAV capsid protein is present in VP1, VP2, and VP3 of the AAV capsid.

38. The AAV capsid of claim 33, wherein the AAV capsid is chimeric.

39. The AAV capsid of claim 33, wherein 60 copies of the AAV capsid protein are assembled into the AAV capsid.

40. The AAV capsid of claim 33 that is isolated and purified.

41. Use of the AAV capsid of claim 33 in the manufacture of a pharmaceutical formulation for treating a disease or a condition of the liver, the pharmaceutical formulation further

comprising a pharmaceutically acceptable carrier, wherein the pharmaceutical formulation is to be intravenously administered.

42. The use of claim 41, wherein the pharmaceutical formulation further comprises a therapeutic agent.
43. A recombinant vector comprising a nucleic acid encoding the AAV capsid protein of any one of claims 1-42.
44. A kit comprising:
 - a) a first vector comprising the recombinant vector of claim 43;
 - b) a second vector encoding a helper virus protein; and
 - c) a third vector comprising a therapeutic nucleic acid encoding a therapeutic gene expression product.
45. The AAV capsid protein of any one of claims 1-42 for use in therapy.
46. Use of the AAV capsid protein of any one of claims 1-42 in the manufacture of a medicament for treating a disease or a condition of a central nervous system or a liver of the subject.
47. A method of manufacturing a recombinant AAV particle from the AAV capsid of any one of claims 1-42, the method comprising:
 - a) introducing into a cell a nucleic acid comprising:
 - i. a first nucleic acid sequence encoding a therapeutic gene expression product;
 - ii. a second nucleic acid sequence encoding a recombinant viral genome comprising a capsid (*Cap*) gene modified to express the AAV capsid of any one of claims 1-42; and
 - iii. a third nucleic acid sequence encoding an AAV helper virus genome; and
 - b) assembling the recombinant AAV particle, the recombinant AAV particle comprising the AAV capsid encapsidating the first nucleic acid.
48. A method of manufacturing a recombinant AAV particle, the method comprising:
 - a) providing a recombinant AAV genome comprising:
 - i. an AAV capsid gene, and

- ii. a recognition sequence for a Cre recombinase, wherein the recognition sequence facilitates a recombinase-dependent change that is detectable, and wherein the recombinase recognition sequence comprises two Cre-recognition sites;
 - b) transfecting a population of cells expressing the Cre recombinase with the recombinant AAV genome, whereby the Cre recombinase induces a recombination event to generate the recombinase-dependent change in the recombinant AAV genome, and wherein the recombinase-dependent change comprises an inversion of the sequence that is flanked by the Cre-recognition sites;
 - c) detecting an increased rate of the recombinase-dependent change a target cell in the population of cells;
 - d) detecting a decreased rate of the recombinase-dependent change in an off-target cell in the population of cells; and
 - e) identifying a recombinant AAV genome generated by the recombinase-dependent change, wherein said identified rAAV genome comprises the inversion, and wherein said identified recombinant AAV genome encodes an AAV capsid particle characterized having an increased specificity for the target cell and a decreased specificity for the off-target cell.
49. The method of claim 48, wherein the off-target cell is a hepatocyte.
50. The method of claim 48, wherein the target cell is a cell selected from the group consisting of a neuron, a glial cell, an oligodendrocyte, an ependymal cell, an astrocyte, a Schwann cell, a satellite cell, and an enteric glial cell.