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(71) Applicants: **NEUEXCELL THERAPEUTICS (SUZHOU) CO., LTD.** [CN/CN]; 218 Xinghu Street, Building B1, Floor 8, Suzhou Industrial Park, Suzhou, Jiangsu 215000 (CN). **NEUEXCELL THERAPEUTICS INC.** [US/US]; 401 N Broad St. Suite M125, Philadelphia, PA 19108 (US).

(72) Inventors: **XU, Jie**; 218 Xinghu Street, Building B1, Floor 8, Suzhou Industrial Park, Suzhou, Jiangsu 215000 (CN). **SHENG, Jian**; 218 Xinghu Street, Building B1, Floor 8, Suzhou Industrial Park, Suzhou, Jiangsu 215000 (CN). **CHEN, Yuchen**; 218 Xinghu Street, Building B1, Floor 8, Suzhou Industrial Park, Suzhou, Jiangsu 215000 (CN). **GUO, Cangzhi**; 218 Xinghu Street, Building B1, Floor 8, Suzhou Industrial Park, Suzhou, Jiangsu 215000 (CN). **ZHOU, Kai**; 218 Xinghu Street, Building B1, Floor 8, Suzhou Industrial Park, Suzhou, Jiangsu 215000 (CN). **JIN, Sen**; 218 Xinghu Street, Building B1, Floor 8, Suzhou Industrial Park, Suzhou, Jiangsu 215000 (CN). **LIU, Meng**; 218 Xinghu Street, Building B1, Floor 8, Suzhou Industrial Park, Suzhou, Jiangsu 215000 (CN). **CHEN, Ming**; 218 Xinghu Street, Building B1, Floor 8, Suzhou Industrial Park, Suzhou, Jiangsu 215000 (CN).

(74) Agent: **AFD CHINA INTELLECTUAL PROPERTY LAW OFFICE**; Tower B, 21st Fl. Suite 2108, 38 Xueqing Road, Haidian District, Beijing 100083 (CN).

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(54) Title: GENE THERAPY COMPOSITIONS AND METHODS FOR TREATING GLIOMA

(57) Abstract: Provided herein are methods and compositions for treating glioma in a subject in need thereof. The method comprises administering to the subject a recombinant AAV comprising a self-complementary genome encoding NeuroD1 alone or in combination with other therapeutic agents. Pharmaceutical formulation comprising the recombinant AAV encoding NeuroD1 is also provided.



WO 2025/108407 A2

Description

Title of Invention: GENE THERAPY COMPOSITIONS AND METHODS FOR TREATING GLIOMA

[] CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to International Patent Application No. : PCT/CN2023/133705 filed on November 23, 2023, the content of which is incorporated by reference in its entirety.

SEQUENCE LISTING

[0002] This application contains a computer readable Sequence Listing which has been submitted in XML file format with this application, the entire content of which is incorporated by reference herein in its entirety. The Sequence Listing XML file submitted with this application is entitled "14770-063-228_SEQLISTING. xml" , was created on November 18, 2024, and is 104, 552 bytes in size.

1. FIELD

[0003] The present disclosure generally relates to compositions and methods for treating glioma.

The method comprises administering a therapeutically effective amount of a pharmaceutical composition

comprising a self-complementary AAV (scAAV) vector encoding a transcriptional factor, wherein the transcriptional factor comprises NeuroD1.

2. BACKGROUND

[0004] A glioma is a type of tumor found in the central nervous system that originates from glial stem or precursor cells. Glial cells are a specific cell type found throughout the nervous system. Gliomas are primarily located in the brain and occasionally in the spinal cord. The occurrence of gliomas varies according to different subtypes and age groups. Excluding instances where other cancers spread to the central nervous system, gliomas constitute 26% of all primary brain tumors and make up 81% of malignant brain tumors. Incidence rates show around 6.6 cases per 100,000 people annually, rising to 2.94 cases per 100,000 individuals below the age of 14. The median age for glioma development ranges from 12 to 65 years, depending on the specific subtype. The prevalent glioma in those under 14 is pilocytic astrocytoma, accounting for 34.4% of all gliomas in this age group. Among adults, glioblastoma (GBM) is the most frequent, comprising 56.6% of all gliomas.

[0005] Gliomas can exert pressure on brain regions where they form, leading to diverse symptoms including headaches, nausea, cognitive decline, seizures, impaired gait, language difficulties (aphasia) , numbness, weakness on one side of the

body (hemiparesis) , altered vision, and personality changes. The conventional approach to treating gliomas typically combines neurosurgery, radiation therapy, and chemotherapy. Nevertheless, the average survival time for glioblastoma patients ranges from 12 to 18 months; merely 25% of patients survive beyond one year, and a mere 5% survive past five years. The development of potential new treatments for gliomas is a very urgent need in medicine. This present disclosure meets this need.

3. SUMMARY

- [0006] Glioma is the malignancy of the central nervous system caused by uncontrolled division of glial cells. Normal glial cells are present in a large quantity in the nervous system and function to nourish neurons and form supporting matrix surrounding them. Unlike neurons, which are terminally differentiated and do not divide, glial cells can undergo cell division cycles, making them prone to tumor formation.
- [0007] Disclosed herein is a gene therapy strategy, and related compositions and methods, which treats glioma by introducing into the cancerous cells a transgene encoding a NeuroD1 protein, where the transgene is overexpressed and induces trans-differentiation of cancerous cells into neurons in situ. It was observed that overexpression of NeuroD1 in healthy glial cells can lead to the manifestation of diverse neuronal phenotypes by the glial cells, suggesting that NeuroD1 may have the potential to reprogram the cell fate of healthy glial cells. Guo et al. *Cell Stem Cell* 14: 188-202 (2014) .
- [0008] Certain in vitro studies also revealed a similar role of NeuroD1 in cultured glioma cells. For example, in one study, cultured human U251 cells were transduced with a retroviral vector encoding NeuroD1, and the majority of virally-infected U251 cells showed a neuronal morphology and expressed immature neuronal markers such as β 3-tubulin (Tuj1) 20 days post transduction. Wang et al. *Cancer Biol Med.* 2021 Mar 23; 18 (3) : 860-874. However, employing the cell fate reprogramming strategy for glioma treatment in patients continues to encounter a myriad of challenges. Evident among these obstacles is the imperative to attain a substantial efficiency in the in vivo transduction of afflicted cells, along with an efficient cancer-to-neuron conversion rate. This convergence of efficiencies is essential to mitigate the potential for cancer cell persistence and subsequent relapse. The vectors responsible for encoding and delivering the NeuroD1 encoding transgene significantly impact such efficiencies. For example, in another study, after transducing cultured human U251 cells with a lentiviral vector encoding NeuroD1, only a small percentage of about 5% cells exhibited positive staining for Tuj1 and displayed some neuronal morphology (WO 2022/052964) , rendering the method inadequate for purpose of treating glioma.

- [0009] In one aspect, provided herein are therapeutic nucleic acid encoding a NeuroD1 polypeptide. In some embodiments, the therapeutic nucleic acids are artificial genomes encoding a NeuroD1 polypeptide. Particularly, in some embodiments, provided herein is a single-stranded self-complementary nucleic acid molecule encoding a NeuroD1 polypeptide, wherein the nucleic acid molecule comprises an expression cassette comprising a coding sequence and one or more regulatory elements operably linked to the coding sequence of the NeuroD1 polypeptide. In related aspects, provided herein are also recombinant adeno-associated virus (rAAV) comprising the artificial genomes as described herein. In related aspects, methods and materials (e.g., plasmids and cells) for production of the recombinant AAVs are also provided.
- [0010] In another aspect, provided herein are pharmaceutical compositions comprising the recombinant AAVs as described herein. Relatedly, in another aspect, provided herein are methods for preventing or treating glioma using the therapeutic nucleic acids, recombinant AAV or pharmaceutical compositions containing the recombinant AAVs as described herein. In yet a related aspect, provided herein are uses of the therapeutic nucleic acids, recombinant AAVs or pharmaceutical compositions containing the recombinant AAV described herein for the prevention or treatment of glioma.
- [0011] The recombinant AAV compositions and related methods according to the present disclosure can achieve in vivo transduction of glioma cells at efficiency of at least 50% in a cell line-derived xenograft (CDX) model, which closely mimic real patient's tumor state and response. The infected glioma cells trans-differentiate in vivo into neuron or neuron-like cells that exhibit one or more neuronal phenotypes, including for example, the expression of neuronal markers, loss of glial markers, and stop cell proliferation. Upon intratumorally administration of the recombinant AAV as described herein or pharmaceutical composition thereof, the tumor size significantly reduced in vivo (for at least 60%) , resulting in significantly prolonged lifespan and extended survival rate of the subject.

4. BRIEF DESCRIPTION OF THE FIGURES

- [0012] FIG. 1 illustrates the triple transfection procedure for producing scAAV particles from a packaging HEK293 cell line. HEK cells were co-transfected with the plasmid encoding the transgene of interest (GOI plasmid) , a rep-cap packaging plasmid that provides the viral rep and cap gene function and aids the production of AAVs from artificial genomes lacking functional rep and/or cap gene sequences, a helper plasmid encoding adenovirus regions (VA, E2A and E4) that mediate AAV vector replication. In the GOI plasmid, the transgene is placed under control of regulatory elements such as a promoter and a poly-A signal. The transgene expression cassette is flanked by two AAV inverted terminal repeat (ITR) sequences. The 3' ITR is mutated to remove terminal resolution site (trs) so that the replication continues to the displaced strand

and produce a self-complimentary genome in the virion. The self-complimentary genome is a single-stranded molecule, which upon entry of a host cell, can fold back upon itself to form a double-stranded hairpin structure with a ITR in the middle and two open-ended ITRs at the two ends (right bottom illustration

- [0013] FIG. 2A illustrates an example of a self-complimentary AAV genome. Particularly, the functional elements along the self-complimentary AAV genome, including, from the 5' to 3' order, (a) a full-length AAV2 left (5') ITR sequence, (b) a CMV promoter including CMV enhancer and CMV core promoter sequences, (c) a chimeric intron sequence, (d) a NeuroD1 coding sequence, (e) a polyadenylation signal, (f) a AAV2 right (3') ITR sequence with the terminal resolution site (trs) deleted, and (g) a reverse complementary sequence of the above (a) to (e) (element (g) is not shown in the bottom panel) .
- [0014] FIG. 2B illustrates another example of a self-complimentary AAV genome. Particularly, the functional elements along the self-complimentary AAV genome, including, from the 5' to 3' order, (a) a AAV2 right (3') ITR sequence with the terminal resolution site (trs) deleted, (b) a CMV promoter including CMV enhancer and CMV core promoter sequences, (c) a chimeric intron sequence, (d) a NeuroD1 coding sequence, (e) a polyadenylation signal, (f) a full-length AAV2 left (5') ITR sequence, (g) a reverse complementary sequence of the above (b) to (f) (element (g) is not shown in the bottom panel) .
- [0015] FIG. 2C shows the sequence alignment of NeuroD1 proteins from various species, including mouse, zebrafish, human, rat, chicken, cattle, hamster, pig, frog, dog, chimpanzee and sheep.
- [0016] FIG. 3 shows the study design for examining in vivo therapeutic efficacy of a scAAV vector encoding NeuroD1 (scAAV-NeuroD1) according to the present disclosure. On day 1, glioblastoma cells were transplanted into the striatum of BALB/c nude mouse brains using stereotactic intracranial injection to establish the orthotopic CDX glioma model. On each of Day 3, 8 and 13 post tumor cell transplantation (DPT) , a formulation containing scAAV-NeuroD1 virus was injected into the tumor. The control group received the same volume of PBS, and the animals in both groups were handled similarly otherwise. A group of mice were sacrificed, and brain tissues were harvested at 14 DPT for immunohistochemistry (IHC) studies. Another group of animals were monitored until they reached the endpoint of life.
- [0017] FIG. 4A shows GFP fluorescence emitted by glioma cell culture (U87MG) transduced in vitro with one of four different AAV serotype virus (ssAAV9-GFAP-GFP, ssAAV6-GFAP-GFP, scAAV9-CMV-GFP, scAAV6-CMV-GFP) , respectively, imaged at 24 hours, 48 hours, 72 hours, 12 hours and 168 hours post transduction. FIG. 4B is a quantification of the fluorescent signal intensity in the images of FIG. 4A.

- [0018] FIG. 4C shows GFP fluorescent images of a U87MG-Luc sphere model treated with scAAV6-CMV-GFP or scAAV9-CMV-GFP. At 99ms exposure time, cells treated with scAAV6-CMV-GFP emitted a strong GFP signal, where GFP signal was undetectable in cells treated with scAAV9-CMV-GFP. At 400ms exposure time, weak GFP signal was detected in cells treated with scAAV9-CMV-GFP.
- [0019] FIG. 5A shows fluorescent imaging of a CDX tumor injected with scAAV6-GFP viral preparation. Tumor cells in the injected area (in the dashed circle) emitted green fluorescence, while cells outside this infected area stained blue (DAPI for nuclei). Scale bar indicates 1000 μ m.
- [0020] FIG. 5B is a close-up image of the AAV infected area, showing a majority of tumor cells in this injected region expressed GFP. Scale bar indicates 100 μ m.
- [0021] FIG. 6 is immunocytochemistry staining of NeuroD1 (purple) and DAPI (blue for nuclei) in cultured U87MG-Luciferase cells 8 days after transduction with scAAV6-NeuroD1 (right) or a negative control vector AAV6-NeuroD1-Null (right, no NeuroD1 expression).
- [0022] FIG. 7 shows quantitation of cell viability in cultures of different glioblastoma cells (U87-Luc, U251-Luc and GL261-Luc) after treatment with ten-fold serial diluted scAAV6-NeuroD1 preparations (MOI: 10^6 to 10^3). Viability of cells treated with PBS was set as 100%.
- [0023] FIG. 8 shows in the right panel a quantitation of the average sphere formation numbers (from three duplicated experiments) in U87MG-Luc cells treated with PBS, scAAV6-NeuroD1 and scAAV9-NeuroD1, respectively. Representative bright-field images of individual cell spheres are shown in the left panel.
- [0024] FIG. 9 shows the percentage proliferation of patient-derived glioma cell lines (BN2276, BN2338 and BN9224) treated with scAAV6-NeuroD1 at 9 different dosages (viral genome (vg) /ml). Proliferation of control groups of untreated cells were set as 100%.
- [0025] FIG. 10A shows the expression of NeuN, NeuroD1 and HuNu as visualized by immunostaining of brain slices of an orthotopic U87MG-Luc transplantation model 14 days after treated with PBS (negative control) or scAAV6-NeuroD1.
- [0026] FIG. 10B shows the quantitation of NeuroD1 expression level (RNA) using GAPDH as reference by RT-qPCR.
- [0027] FIG. 11 shows inhibitory effect of scAAV6-NeuroD1 on tumor growth in the U87MG-Luc GBM mouse model. FIG. 11A shows DAPI immunostaining visualizing all nuclei in the exemplary brain sections contrasting animals received PBS (upper panel) and animals received scAAV6-NeuroD1 treatment (lower panel). FIG. 11B shows H&E staining visualizing histological morphology of the tumor in animals received PBS (upper panel) and animals received scAAV6-NeuroD1 treatment. FIG.

11C shows quantitation of the tumor size in animals received PBS (left bar) and animals received scAAV6-NeuroD1 treatment (right bar) .

[0028] FIG. 12 shows comparison of in vivo imaging (IVIS) of U87MG-Luc cells in the brain of U87MG-Luc orthotopic CDX model that received PBS (negative control) and scAAV6-NeuroD1.

[0029] FIG. 13 showed immunostaining of Ki67 (middle panel) and DAPI (all nuclei, right panel) of brain slices of glioma CDX model on 14 DPT. The negative control group received PBS and the treatment group received scAAV6-NeuroD1 on days 3, 8, and 13 DPT, respectively. Mice were sacrificed on 14 DPT and subjected to immunohistochemistry (IHC) analysis for the nuclear protein Ki67 as an indicator of tumor aggressiveness. As shown, the expression of ki67 in the control group was higher as measured in both area and intensity as compared to those in the treatment group.

[0030] FIG. 14 shows qPCR analysis of Ki67 and NeuroD1 expression levels in the brains of glioma CDX model mice in the treatment group that received scAAV6-NeuroD1 and control group that received PBS.

[0031] FIG. 15A shows immunostaining of microglia/macrophage marker Iba1 in brain slice of glioma CDX model mice that received scAAV6-NeuroD1 or PBS (control) 14 DPT. DAPI stains for all nuclei. FIG. 15B shows quantitative analysis of Iba1 fluorescence intensity between the treatment and control groups.

[0032] FIG. 16 shows the change of body weight of glioma CDX mice received scAAV6-NeuroD1 or PBS (control) . As shown, body weight of the treatment group remained normal for an extended time as compared to the control group.

[0033] FIG. 17 shows life span of glioma CDX model mice that received scAAV6-NeuroD1 or PBS (control) . The median survival of control animals was 16 DPT (n=21) , while that of scAAV6-NeuroD1 treatment group reached 34 DPT (n=20) , ($p < 0.0001$) .

[0034] FIG. 18 shows comparison of survival rate of groups of glioma CDX model mice received treatment with scAAV6-NeuroD1, its serotype comparative scAAV9-NeuroD1, or a comparative scAAV9-Ngn2. The scAAV6-NeuroD1 treated group had the longest life span comparing to the other groups.

[0035] FIG. 19 shows survival rate of groups of glioma CDX model mice treated with scAAV6-NeuroD1 alone, Temozolomide alone, or a combination of scAAV6-NeuroD1 and Temozolomide. The combination therapy exhibited synergistic effect over either monotherapy.

[0036] FIG. 20 shows dose-dependent efficacy study of scAAV6-NeuroD1 on survival.

[0037] FIG. 21 shows in vivo efficacy of scAAV6-NeuroD1 in PDX models of glioblastoma. Tumor growth in NOD/SCID mice bearing GBM BN2276 xenografts treated with 5E11 vg/injection scAAV6-NeuroD1 or PBS (control) . Data are presented as mean $\pm$ SD.

- [0038] FIG. 22 shows transcriptome change in GBM cell U87 treated with scAAV6-NeuroD1 versus with empty AAV capsid. Gene Ontology (GO) term enrichment analysis of differentially expressed genes in NXL-004-treated GBM cells comparing with empty AAV capsid treated cells 3 days post-infection. The bar graph highlights significant downregulation of genes involved in cell division, mitotic cell cycle, DNA replication. These changes are indicative of inhibition of cell division and proliferation by NXL-004 treatment.
- [0039] FIG. 23 shows transcriptome change in GBM cell U87 treated with scAAV6-NeuroD1 versus with PBS. Gene Ontology (GO) term enrichment analysis of differentially expressed genes in GBM cells treated with NXL-004 comparing with control (PBS) at 3 days post-infection. The bar graph highlights significant downregulation of genes involved in cell division, mitotic cell cycle, DNA replication. These changes are indicative of inhibition of cell division and proliferation by NXL-004 treatment.
- [0040] FIG. 24 shows transcriptome change in GBM cells U251 treated with scAAV6-NeuroD1 comparing to cells treated with empty AAV capsid. Comparing RNA-seq results of GBM cell U251 treated with scAAV6-NeuroD1 or empty AAV capsid showed upregulation of the genes for neural development and activity.
- [0041] FIG. 25 shows in vivo infection of scAAV6 in normal mouse brain at different doses.
- [0042] FIGS. 26A and 26B show NeuroD1 expression level and prognosis correlation in GBM patients. NeuroD1 expression inversely correlated with poor prognosis of GBMLGG patients. FIG. 26A shows the Kaplan-Meier plot of overall survival (OS) and FIG. 26B shows the progression-free interval (PFI) in glioma patients from TCGA lower grade glioma and glioblastoma (GBMLGG) cohort, stratified by NeuroD1 expression (gene expression RNAseq: IlluminaHiSeq). The NeuroD1 expression levels were classified into two groups: The low NeuroD1 group had a relative expression value < the cohort mean expression value, while the high NeuroD1 group had a relative expression value > the cohort mean expression value. The log-rank test was used to compare differences between groups (n=694). Data source: UCSC Xena.
- [0043] FIGS. 27A and 27B show NeuroD1 expression level in GBMLGG patients and IDH1 status correlation. FIG. 27A shows the relative expression of NeuroD1 in female and male of glioma patients within the TCGA GBMLGG cohort (n=694). Data are shown as mean $\pm$ SD. P=0.6025 (by two-tailed unpaired t-test). FIG. 27B shows the relative expression of NeuroD1 in IDH1 wild-type (WT) and IDH1 mutation (MT) of glioma patients in the TCGA GBMLGG cohort (n=125). Data are shown as mean $\pm$ SD. P=0.0012 (by two-tailed unpaired t-test).
- [0044] FIGS. 28A, 28B and 28C show the correlation between the NeuroD1 expression level and prognosis in GBM patients. Overall survival data of NeuroD1 in glioma patients from three CGGA cohorts: mRNAseq_325 (FIG. 28A), mRNAseq_693

(FIG. 28B) , and mRNA-array_301 (FIG. 28C) . The NeuroD1 expression levels were classified into low and high groups by the CGGA website. Cohort: mRNAseq_325, n=222; cohort: mRNAseq_693 , n=404; cohort: mRNA-array_301, n=262. Data source: Chinese Glioma Genome Atlas.

5.DETAILED DESCRIPTION

- [0045] It has been observed that overexpression of several selected transcription factors in healthy glial cells can result in reprogramming of the cell fate, converting differentiated glial cells into cells exhibiting neuronal phenotypes in vitro. Inventors of the present disclosure has made the surprising discovery that in glial cells undergoing various stages of pathogenic neoplasm, such as glioma cells, overexpressing a single transcription factor NeuroD1 can effectively reprogram the cancer cells into neurons or neuron-like cells both in vitro and in vivo. Furthermore, utilizing an efficient strategy to deliver and express a transgene encoding NeuroD1 in subject suffering from glioma, the inventors of the present disclosure discovered that the therapy was effective in halting cancer cell proliferation, reducing the glioma tumor size, and increasing life span of the afflicted subjects.
- [0046] Recombinant AAV-based gene therapy represents a highly promising therapeutic strategy for introducing therapeutic gene and gene products into afflicted cells. Traditional gene delivery using recombinant AAV entails the incorporation of linear single-stranded DNA molecules into virions, which upon entering into a host cell, undergoes replication to generate complementary strands. The present disclosure also based partially upon the discovery that utilizing recombinant AAV comprising a self-complementary genome can bypass the step of double strand synthesis, resulting in much more swift transgene expression that is independent upon cellular replication machinery, and significantly enhanced efficiency of the delivery and expression of the transgene. Furthermore, the present disclosure also based partially upon the discovery of selected nucleotide sequences that can be used as AAV genome-compatible elements to significantly enhance the level of expression of a transgene carried by the recombinant AAV genome in glioma cells. Furthermore, the present disclosure also based partially on the discovery that recombinant AAV comprising a AAV serotype 6 (AAV6) capsid is more efficient in transducing glioma cells.
- [0047] Accordingly, in one aspect of the present disclosure, provided herein are regulatory elements (e.g., regulatory elements disclosed in Section 5.3.2 (Untranslated Regions) of the present disclosure) that confer a high level of expression of an encoding nucleic acid in glial cells, including glial cells that are undergoing various stages of pathogenic neoplasm, such as glioma cells. In a related aspect of the present disclosure, provided herein are also expression cassettes, (e.g., expression cassettes disclosed in Section

5.3 (NeuroD1 Expression Cassette) of the present disclosure) , artificial genome for recombinant AAV (e.g., self-complementary AAV genomes disclosed in Section 5.4 (self-complementary AAV (scAAV) genomes) of the present disclosure) recombinant AAV (e.g., recombinant AAV disclosed in Section 5.5 (Recombinant scAAV vectors) of the present disclosure) , plasmids and host cells (e.g., plasmids and host cells disclosed in Section 5.6 (Methods and Compositions for Making Recombinant AAV) of the present disclosure) . The present disclosure further provides pharmaceutical compositions and kits (e.g., pharmaceutical compositions and kits disclosed in Section 5.7 (Pharmaceutical Composition and Kit) of the present disclosure) comprising the presently disclosed recombinant AAV. Methods of using the presently disclosed recombinant AAV described herein (e.g., methods of Treatment disclosed in Section 5.8 (Method of Treatment) of the present disclosure) are also provided by the present disclosure.

5.1 General Techniques

- [0048] Techniques and procedures described or referenced herein include those that are generally well understood and/or commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* (3d ed. 2001) ; *Current Protocols in Molecular Biology* (Ausubel et al. eds., 2003) .

5.2 Terminology

- [0049] Unless described otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art. For purposes of interpreting this specification, the following description of terms will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. All patents, applications, published applications, and other publications are incorporated by reference in their entirety. In the event that any description of terms set forth conflicts with any document incorporated herein by reference, the description of term set forth below shall control.

- [0050] The term “AAV” or “adeno-associated virus” refers to a Dependoparvovirus within the Parvoviridae genus of viruses. The AAV can be an AAV derived from a naturally occurring “wild-type” virus, or a recombinant AAV (rAAV) that is derived from a naturally occurring AAV, but having all or part of the AAV genome replaced with heterologous nucleotide sequences (e.g., expression cassettes disclosed in Section 5.3 (NeuroD1 Expression Cassette) of the present disclosure comprising a coding sequence and regulatory elements) . In certain embodiments, the rAAV comprises an AAV genome (e.g., an artificial genome) in which part or all of the Rep (Replication) and/or Cap (Capsid) genes have been replaced with heterologous nucleotide sequences, such as a transgene. In the absence of Rep proteins, the heterologous nucleotide sequences

encoded within the rAAV can persist as episomes in the nucleus of transduced cells and does not integrate into host genomes. In certain embodiments, the rAAV further comprises a capsid comprising capsid proteins encoded by a naturally occurring or non-naturally occurring Cap gene. In certain embodiments, the non-naturally occurring Cap gene encodes a capsid protein comprising an insertion, deletion, or modification of the amino acid sequence of the naturally occurring capsid protein. For example, a rAAV can have an artificial genome packaged in a capsid having a viral protein 1 (VP1), viral protein 2 (VP2), or viral protein 3 (VP3), where the VP1 sequence is different from the wild-type sequence, while VP2 and VP3 both have wild-type sequences. As used herein, a rAAV that carries a heterologous transgene of interest in the genome is sometimes referred to as a “AAV vector.”

- [0051] The term “rep-cap packaging plasmid” refers to a plasmid that provides the viral rep and cap gene function and aids the production of AAVs from artificial genomes lacking functional rep and/or cap gene sequences.
- [0052] The term “cap gene” refers to the nucleic acid sequences that encode capsid proteins that form or help form the capsid of the virus. In some embodiments, in a recombinant AAV virion, the capsid protein contains VP1, VP2, and/or VP3.
- [0053] The term “rep gene” refers to the nucleic acid sequences that encode the non-structural proteins needed for replication and production of virus.
- [0054] The term “polynucleotide” or “nucleic acid,” as used interchangeably herein, refers to polymers of nucleotides of any length and includes, e.g., DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. Nucleic acid can be in either single- or double-stranded forms. As used herein and unless otherwise specified, “nucleic acid” also includes nucleic acid mimics such as locked nucleic acids (LNAs), peptide nucleic acids (PNAs), and morpholinos. “Oligonucleotide,” as used herein, refers to short synthetic polynucleotides that are generally, but not necessarily, fewer than about 200 nucleotides in length. The terms “oligonucleotide” and “polynucleotide” are not mutually exclusive. The description above for polynucleotides is equally and fully applicable to oligonucleotides. Unless specified otherwise, the left-hand end of any single-stranded polynucleotide sequence disclosed herein is the 5' end; the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA transcript that are 5' to the 5' end of the RNA transcript are referred to as “upstream sequences”; sequence

regions on the DNA strand having the same sequence as the RNA transcript that are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences."

- [0055] As used herein, the term "wild-type" refers to organisms, cells, genes, proteins, oligonucleotides, and the like that are found in Nature and are unchanged relative to these components found in Nature (native or in the wild) .
- [0056] As used herein, the term "non-naturally occurring" when used in reference to a nucleic acid molecule as described herein is intended to mean that the nucleic acid molecule is not found in nature. A non-naturally occurring nucleic acid encoding a protein (e.g., NeuroD1) contains at least one genetic alternation or chemical modification not normally found in a naturally occurring nucleic acid, including a wild-type nucleic acid. Genetic alterations include, for example, modifications to an expressible nucleic acid sequences encoding heterologous peptides or polypeptides, other nucleic acid additions, nucleic acid deletions, nucleic acid substitution, and/or other functional disruption of a coding sequence. Such modifications include, for example, modifications in the coding regions and functional fragments thereof, for heterologous, homologous or both heterologous and homologous polypeptides. Additional modifications include, for example, modifications in non-coding regulatory regions in which the modifications alter expression of a gene or operon. Additional modifications also include, for example, incorporation of a nucleic acid sequence into a vector, such as a plasmid or an artificial chromosome. Chemical modifications include, for example, one or more functional nucleotide analog as described herein.
- [0057] An "isolated nucleic acid" is a nucleic acid, for example, an RNA, DNA, or a mixed nucleic acids, which is substantially separated from other genome DNA sequences as well as proteins or complexes such as ribosomes and polymerases, which naturally accompany a native sequence. An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an "isolated" nucleic acid molecule, such as a plasmid, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In a specific embodiment, one or more nucleic acid molecules encoding a polypeptide as described herein are isolated or purified. The term embraces nucleic acid sequences that have been removed from their naturally occurring environment, and includes recombinant or cloned DNA or RNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure molecule may include isolated forms of the molecule.
- [0058] The term "encoding nucleic acid," "nucleic acid encoding" or other grammatical equivalents thereof as it is used in reference to nucleic acid molecule encompasses (a)

a nucleic acid molecule in its native state or when manipulated by methods well known to those skilled in the art that can be transcribed to produce mRNA which is then translated into a peptide and/or polypeptide, and (b) the mRNA molecule itself. The antisense strand is the complement of such a nucleic acid molecule, and the encoding sequence can be deduced therefrom. The term “coding region” or “coding sequence” refers to a portion in an encoding nucleic acid sequence that is translated into a peptide or polypeptide. The term “intron” refers to the portion of an encoding nucleic acid that is not transcribed into an mRNA. The term “untranslated region” or “UTR” refers to the portion of an encoding nucleic acid that is not translated into a peptide or polypeptide. Depending on the orientation of a UTR with respect to the coding region of a nucleic acid molecule, a UTR is referred to as the 5' -UTR if located to the 5' -end of a coding region, and a UTR is referred to as the 3' -UTR if located to the 3' -end of a coding region. In some embodiments, an encoding nucleic acid described herein is a transgene encoding a NeuroD1 polypeptide. In some embodiments, the transgene encoding a NeuroD1 polypeptide is a DNA molecule.

- [0059] An encoding nucleic acid can be mono-cistronic or multi-cistronic. A “mono-cistronic sequence” refers to a polynucleotide that comprises coding sequence for a single peptide or polypeptide chain. A “multi-cistronic sequence” refers to a polynucleotide that comprises coding sequences for two or more peptide and/or polypeptide chains.
- [0060] The term “mRNA” as used herein refers to a message RNA molecule comprising one or more open reading frame (ORF) that can be translated by a cell or an organism provided with the mRNA to produce one or more peptide or protein product. The region containing the one or more ORFs is referred to as the coding region of the mRNA molecule. In certain embodiments, the mRNA molecule further comprises one or more untranslated regions (UTRs) . In certain embodiments, the mRNA is or is part of a linear RNA molecule. In other embodiments, the mRNA is or is part of a circular RNA molecule.
- [0061] In certain embodiments, the mRNA is a monocistronic mRNA that comprises only one ORF. In certain embodiments, the monocistronic mRNA encodes a peptide or protein comprising at least one epitope of a selected polypeptide (e.g., transcription factor) . In other embodiments, the mRNA is a multicistronic mRNA that comprises two or more ORFs. In certain embodiments, the multicistronic mRNA encodes two or more peptides or proteins that can be the same or different from each other.
- [0062] As used herein, the term “ribosomal skipping element” refers to a nucleotide sequence capable of causing generation of two polypeptide chains from translation of one RNA molecule. In some embodiments, the ribosomal skipping element can terminate translation of the first polypeptide chain and re-initiating translation of

the second polypeptide chain from the RNA molecule. In alternative embodiments, the ribosomal skipping element encodes a protease cleavage site in the polypeptide encoded by the RNA molecule, so that the polypeptide can be cleaved by an intrinsic protease activity of its own, or by another protease in its environment to produce two polypeptide chains. In specific embodiments, the ribosomal skipping element encodes thosea-asigna virus 2A peptide (T2A) , porcine teschovirus-1 2 A peptide (P2A) , foot-and-mouth disease virus 2 A peptide (F2A) , equine rhinitis A vims 2A peptide (E2A) , cytoplasmic polyhedrosis vims 2A peptide (BmCPV 2A) , or flacherie vims of B. mori 2A peptide (BmIFV 2A) .

[0063] The term “nucleobases” encompasses purines and pyrimidines, including natural compounds adenine, thymine, guanine, cytosine, uracil, inosine, and natural or synthetic analogs or derivatives thereof.

[0064] The term “functional nucleotide analog” as used herein refers to a modified version of a canonical nucleotide A, G, C, U or T that (a) retains the base-pairing properties of the corresponding canonical nucleotide, and (b) contains at least one chemical modification to (i) the nucleobase, (ii) the sugar group, (iii) the phosphate group, or (iv) any combinations of (i) to (iii) , of the corresponding natural nucleotide. As used herein, “base pairing” encompasses not only the canonical Watson-Crick adenine-thymine, adenine-uracil, or guanine-cytosine base pairs, but also base pairs formed between canonical nucleotides and functional nucleotide analogs or between a pair of functional nucleotide analogs, wherein the arrangement of hydrogen bond donors and hydrogen bond acceptors permits hydrogen bonding between a modified nucleobase and a canonical nucleobase or between two complementary modified nucleobase structures. For example, a functional analog of guanosine (G) retains the ability to base-pair with cytosine (C) or a functional analog of cytosine. One example of such non-canonical base pairing is the base pairing between the modified nucleotide inosine and adenine, cytosine, or uracil. As described herein, a functional nucleotide analog can be either naturally occurring or non-naturally occurring. Accordingly, a nucleic acid molecule containing a functional nucleotide analog can have at least one modified nucleobase, sugar group and/or internucleoside linkage. Exemplary chemical modifications to the nucleobases, sugar groups, or internucleoside linkages of a nucleic acid molecule are provided herein.

[0065] The terms “complement” or “complementary” can be determined by the Watson-Crick base pairing between nucleotides and specifically refers to nucleotides hydrogen bonded to one another with thymine or uracil residues linked to adenine residues by two hydrogen bonds and cytosine and guanine residues linked by three hydrogen bonds. In general, a nucleic acid includes a nucleotide sequence described as having a “percent complementarity” to a specified second nucleotide sequence. For example,

a nucleotide sequence may have 80%, 90%, or 100% complementarity to a specified second nucleotide sequence, indicating that 8 of 10, 9 of 10 or 10 of 10 nucleotides of a sequence are complementary to the specified second nucleotide sequence. For instance, the nucleotide sequence 3' -TCGA-5' is 100% complementary to the nucleotide sequence 5' -AGCT-3' . Further, the nucleotide sequence 3' -TCGA-is 100% complementary to a region of the nucleotide sequence 5' -TTAGCTGG-3' .

[0066] The term “reverse complementary” means two nucleic acid sequences complement to each other when read in opposite directions. A pair of reverse complementary sequences can be in separated nucleic acid molecules or in different regions of a single nucleic acid molecule. In the latter case, the nucleic acid molecule is considered “self-complementary. ” As used herein a “self-complementary” nucleic acid molecule can have at least two regions that are complementary or substantially complementary to each other when read in opposite directions. Under a suitable condition, a pair of reverse-complementary regions are capable of base-pairing with each other to form a double-stranded duplex, and the sequence between the reverse-complementary regions is bend into an unpaired loop. The resulting structure is referred to as a “stem-loop, ” a “hairpin, ” or a “hairpin loop, ” which is a secondary structure found in many self-complementary molecules.

[0067] The terms “duplexed, ” “double-stranded, ” or “hybridized” as used herein refer to multiple nucleic acid molecules or a region of a single nucleic acid molecule (e.g., the stem region in a stem-loop structure) that is formed by hybridization of two single strands of nucleic acids containing complementary sequences. As described herein, a pair of complementary sequences can be fully complementary or partially complementary.

[0068] The terms “hybridization” and “hybridizes” refer to pairing and binding of complementary nucleic acids. Hybridization occurs to varying extents between two nucleic acids depending on factors such as the degree of complementarity of the nucleic acids, the melting temperature, T_m , of the nucleic acids and the stringency of hybridization conditions, as is well known in the art. The term “stringency of hybridization conditions” refers to conditions of temperature, ionic strength, and composition of a hybridization medium with respect to particular common additives such as formamide and Denhardt's solution. Determination of particular hybridization conditions relating to a specified nucleic acid is routine and is well known in the art, for instance, as described in J. Sambrook and D.W. Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press; 3rd Ed., 2001; and F.M. Ausubel, Ed., *Short Protocols in Molecular Biology*, Current Protocols; 5th Ed., 2002. High stringency hybridization conditions are those which only allow hybridization of substantially complementary nucleic acids. Typically, nucleic acids having about

85-100% complementarity are considered highly complementary and hybridize under high stringency conditions. Intermediate stringency conditions are exemplified by conditions under which nucleic acids having intermediate complementarity, about 50-84% complementarity, as well as those having a high degree of complementarity, hybridize. In contrast, low stringency hybridization conditions are those in which nucleic acids having a low degree of complementarity hybridize.

[0069] The term “operably linked” as used herein refers to a nucleic acid sequence in functional relationship with a second nucleic acid sequence. The term “operably linked” encompasses functional connection of two or more nucleic acid sequences, such as a nucleic acid to be transcribed and a regulatory element. The term “regulatory element” as used herein refers to a nucleotide sequence which controls some aspect of the expression of an operably linked nucleic acid coding sequence. Exemplary regulatory elements include an enhancer, such as, but not limited to: woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) ; an internal ribosome entry site (IRES) or a 2A domain; an intron (e.g., a group I intron) ; an origin of replication; a polyadenylation signal (pA) ; a promoter; a transcription termination sequence; and an upstream regulatory domain, which contribute to the replication, transcription, posttranscriptional processing of an operably linked nucleic acid sequence. Those of ordinary skill in the art are capable of selecting and using these and other regulatory elements in an expression vector with no more than routine experimentation.

[0070] The terms “translational enhancer element,” “TEE” and “translational enhancers” as used herein refers to a region in a nucleic acid molecule that functions to promote translation of an operably linked coding sequence of the nucleic acid into a protein or peptide product, such as via cap-dependent or cap-independent translation. A TEE typically locates in the UTR region of a nucleic acid molecule (e.g., mRNA) and enhance the translational level of a coding sequence located either upstream or downstream. For example, a TEE in a 5' -UTR of a nucleic acid molecule can locate between the promoter and the starting codon of the nucleic acid molecule. Various TEE sequences are known in the art (Wellensiek et al. Genome-wide profiling of human cap-independent translation-enhancing elements, *Nature Methods*, 2013 Aug; 10 (8) : 747–750; Chappell et al. PNAS June 29, 2004 101 (26) 9590-9594) . Some TEEs are known to be conserved across multiple species (Pánek et al. *Nucleic Acids Research*, Volume 41, Issue 16, 1 September 2013, Pages 7625–7634) . In particular embodiments, a TEE is a promoter.

[0071] The term “promoter” is a term of art and is used herein to refer to a nucleic acid sequence operably linked to a nucleic acid sequence to be transcribed such as a nucleic acid sequence encoding a NeuroD1 polypeptide as described herein. In some

embodiments, a promoter is positioned upstream of a nucleic acid sequence to be transcribed and provides a site for specific binding by RNA polymerase and other transcription factors.

[0072] In some embodiments, a promoter specifically enhances expression of an operably linked nucleic acid in a given cell type, and such promoter is referred to as a “cell type-specific promoter.” In certain embodiments, a cell type-specific promoter is a glial cell specific promoter. Non-limiting examples of glial cell-specific promoters that can be used in connection with the present disclosure include but are not limited to glial fibrillary acidic protein (GFAP) promoter and aldehyde dehydrogenase 1 family, member L1 (AldhlL1) promoter, a lipocalin 2 (lcn2) promoter, a S100 calcium-binding protein B (S100 β) promoter, a SRY-box transcription factor 9 (Sox9) promoter. A non-limiting example of an NG2 cell-specific promoter is the promoter of the chondroitin sulfate proteoglycan 4 gene, also known as neuron-glia antigen 2 (NG2).

[0073] In alternative embodiments, a promoter generally enhances expression of an operably linked nucleic acid in various different cell types, such as at least 5 different cell types, and such promoter is referred to as an “ubiquitous promoter.” Non-limiting examples of ubiquitous promoters that can be used in connection with the present disclosure include but are not limited to the CAG promoter which combines the cytomegalovirus CMV early enhancer element and chicken beta-actin promoter, a CMV promoter, a ubiquitin promoter, an EF-1a promoter.

[0074] As used herein, an “internal ribosome entry site” or “IRES” refers to an RNA sequence or structural element ranging in size from 10 nt to 1000 nt or more, capable of initiating translation of a polypeptide in the absence of a typical RNA cap structure. An IRES is typically about 500 nt to about 700 nt in length.

[0075] Codon substitution or codon replacement in the context of codon optimization refer to replacing a codon present in a candidate nucleotide sequence (e.g., an mRNA encoding a therapeutic agent) with another codon. Thus, a codon can be substituted in a candidate nucleic acid sequence, for example, via chemical peptide synthesis or through recombinant methods known in the art. Accordingly, references to a “substitution” or “replacement” at a certain location in a nucleic acid sequence (e.g., an mRNA) or within a certain region or subsequence of a nucleic acid sequence (e.g., an mRNA) refer to the substitution of a codon at such location or region with an alternative codon. As used herein, the term “codon-optimized variant” refers to a synonymous nucleotide sequence that encodes the same polypeptide sequence encoded by a candidate nucleotide sequence (e.g., a nucleotide sequence encoding a NeuroD1 polypeptide). Thus, there are no amino acid substitutions in the polypeptide encoded by the codon optimized nucleotide sequence with respect to the polypeptide encoded by the candidate nucleotide sequence. A candidate nucleic acid sequence can be

codon-optimized by replacing all or part of its codons according to a substitution table map. According to the present disclosure, a candidate nucleotide sequence can be codon-optimized, for example, to improve its translation efficacy of the encoded polypeptide. In some embodiments, the candidate nucleotide sequence is codon-optimized for improved translation efficacy after in vivo administration, e.g., administration as part of a recombinant AAV virion.

- [0076] The term “peptide” as used herein refers to a polymer containing between two and fifty (2-50) amino acid residues linked by one or more covalent peptide bond (s) . The terms apply to naturally occurring amino acid polymers as well as amino acid polymers in which one or more amino acid residues is a non-naturally occurring amino acid (e.g., an amino acid analog or non-natural amino acid) .
- [0077] The terms “polypeptide” and “protein” are used interchangeably herein to refer to a polymer of greater than fifty (50) amino acid residues linked by covalent peptide bonds. That is, a description directed to a polypeptide applies equally to a description of a protein, and vice versa. The terms apply to naturally occurring amino acid polymers as well as amino acid polymers in which one or more amino acid residues is a non-naturally occurring amino acid (e.g., an amino acid analog) . As used herein, the terms encompass amino acid chains of any length, including full length proteins (e.g., NeuroD1) .
- [0078] As used herein, the term “NeuroD1 polypeptide” refers to NeuroD1 or a functional derivative of NeuroD1.
- [0079] The term “neurogenic differentiation 1 protein” or “NeuroD1” as used herein, refers to any native NeuroD1 from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats) , unless otherwise indicated. The term encompasses unprocessed NeuroD1 as well as any form of NeuroD1 that results from processing in the cell. The term also encompasses naturally occurring variants of NeuroD1, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human NeuroD1 is
- MTKSYSESGLMGEPQPQGPPSWTDECLSSQDEEHEADKKEDDLETM-
 NAEEDSLRNGGEEDED
 EDLEEEEEEEEEEDDDQKPKRRGPKKKKMTKARLERFKLRRMKANAR-
 ERNRMHGLNAALDNLR KVVPCYSKTQKLSKIETLRLAKNYIWALSEIL-
 RSGKSPDLVSFVQTLCKGLSQPTTNLVAGCLQL NPRTFLPEQNQDMPPHLP-
 TASASFPVHPYSYQSPGLSPPYGTMDSSHVHFVKPPPHAYSAALEPF FES-
 PLTDCTSPSFDGPLSPPLSINGNFSFKHEPSAEFEKKNYAFTMHYPAATLA-
 GAQSHGSIFSGTAA PRCEIPIDNIMSFDSHSHHERVMSAQLNAIFHD (SEQ ID
 NO: 1; GenBank Accession NP_002491.3) . A “full-length” NeuroD1 as used herein refers to the mature, natural length NeuroD1 molecule. For example, full-length human

NeuroD1 refers to a molecule that has 356 amino acids (see e.g., SEQ ID NO: 1) . For example, a functional derivative of NeuroD1 is SEQ ID NO: 4, which has 357 amino acids.

[0080] An ortholog is a gene or genes that are related by vertical descent and are responsible for substantially the same or identical functions in different organisms. For example, mouse NeuroD1 and human NeuroD1 can be considered orthologs for the biological function of regulating neuronal differentiation and neurogenesis. See e.g., Cho, J.H. et al., *Mol. Neurobiol.*, 30: 35-47, 2004; Kuwabara, T. et al., *Nature Neurosci.*, 12: 1097-1105, 2009; and Gao, Z. et al., *Nature Neurosci.*, 12: 1090-1092, 2009. Genes are related by vertical descent when, for example, they share sequence similarity of sufficient amount to indicate they are homologous, or related by evolution from a common ancestor. Genes can also be considered orthologs if they share three-dimensional structure but not necessarily sequence similarity, of a sufficient amount to indicate that they have evolved from a common ancestor to the extent that the primary sequence similarity is not identifiable. Genes that are orthologous can encode proteins with sequence similarity of about 25% to 100% amino acid sequence identity. Genes encoding proteins sharing an amino acid similarity less than 25% can also be considered to have arisen by vertical descent if their three-dimensional structure also shows similarities. Orthologs include genes or their encoded gene products that through, for example, evolution, have diverged in structure or overall activity. For example, where one species encodes a gene product exhibiting two functions and where such functions have been separated into distinct genes in a second species, the three genes and their corresponding products are considered to be orthologs. Those skilled in the art will understand how to identify orthologous genes harboring a biological function of interest. For example, a list of orthologous NeuroD1 genes and encoded NeuroD1 protein sequences can be found on GenBank website: www.ncbi.nlm.nih.gov/gene/4760/ortholog/?scope=89593&term=NEUROD1.

Non-exhaustive examples

of NeuroD1 proteins from various non-human organisms as identified by their respective GenBank accession numbers include *Mus musculus* (house mouse) NP_035024.1, *Danio rerio* (zebrafish) NP_571053.1, *Gallus gallus* (chicken) NP_990251.2, *Bos taurus* (cattle) NP_001096758.1, *Mesocricetus auratus* (golden hamster) XP_005065174.1, *Sus scrofa* (pig) XP_020931169.1, *Xenopus tropicalis* (frog) NP_001090868.1, *Canis lupus familiaris* (dog) XP_005640434.2, *Pan troglodytes* (chimpanzee) XP_001158946.1, *Ovis aries* (sheep) XP_011987527.1. In some embodiments, A group orthologs genes encode protein products that can be considered functional derivatives of one another.

- [0081] NeuroD1 is highly conserved in the vertebrate family. FIG. 2B shows the sequence alignment of NeuroD1 proteins from various species, including mouse, zebrafish, human, rat, chicken, cattle, hamster, pig, frog, dog, chimpanzee and sheep. As shown, at least 95% amino acid residues in the NeuroD1 sequences are conserved across NeuroD1 orthologs from various species.
- [0082] As used herein, the term “Ngn2 polypeptide” refers to Ngn2 or a functional derivative of Ngn2. The term “neurogenin 2” or “Ngn2” as used herein, refers to any native Ngn2 from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses unprocessed Ngn2 as well as any form of Ngn2 that results from processing in the cell. The term also encompasses naturally occurring variants of Ngn2, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human Ngn2 is MFVKSETLELKEEEDVLVLLGSASPALAALTPLSSSADEEEEEEP-GASGGARRQRGAEGQGARG GVAAGAEGCRPARLLGLVHDCKRRPSRAR-AVSRGAKTAETVQRIKKTRRLKANNRERNRMHNL NAALDALREVLPTF-PEDAKLTKIETLRFHNYIWALTETLRLADHCGGGGGGLPGALFSEAVLLSPGGASAALSSSGDSPSPASTWSCTNSPAPSSSVSSNSTSPYSCTLSPASPAGSD-MDYWQPPPPDKHR YAPHLPIARDCI (SEQ ID NO: 35; GenBank Accession: NP_076924.1).
- [0083] A “modification” of an amino acid residue/position refers to a change of a primary amino acid sequence as compared to a starting amino acid sequence, wherein the change results from a sequence alteration involving said amino acid residue/position. For example, typical modifications include substitution of the residue with another amino acid (e.g., a conservative or substantial substitution), insertion of one or more (e.g., generally fewer than 5, 4, or 3) amino acids adjacent to said residue/position, and/or deletion of said residue/position.
- [0084] Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been generally defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys,

Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe. Generally, conservative substitutions in the sequences of the peptides or polypeptides the disclosure do not abrogate the biological activity of interest of the peptide or polypeptide. Amino acid substitutions may be introduced into a polypeptide of interest and the products screened for a desired activity of interest, e.g., retained/improved ability of a NeuroD1 variant in producing one or more neuronal phenotypes in a glia cell, and methods for measuring such desired activity are well-known in the art.

[0085] In contrast, substantial modifications in the biological properties of a polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

[0086] In the context of a peptide or polypeptide, the term “derivative” as used herein refers to a peptide or polypeptide that comprises an amino acid sequence of the peptide or polypeptide, or a fragment of a peptide or polypeptide, which has been altered by the introduction of amino acid residue substitutions, deletions, or additions. The term “derivative” as used herein also refers to a peptide or polypeptide, or a fragment of a peptide or polypeptide, which has been chemically modified, e.g., by the covalent attachment of any type of molecule to the polypeptide. For example, but not by way of limitation, a peptide or polypeptide or a fragment of the peptide or polypeptide may be chemically modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, chemical cleavage, formulation, metabolic synthesis of tunicamycin, linkage to a cellular ligand or other protein, etc. The derivatives are modified in a manner that is different from naturally occurring or starting peptide or polypeptides, either in the type or location of the molecules attached. Derivatives further include deletion of one or more chemical groups which are naturally present on the peptide or polypeptide. Further, a derivative of a peptide or polypeptide or a fragment of a peptide or polypeptide may contain one or more non-classical amino acids. In specific embodiments, a derivative is a functional derivative of the native or unmodified peptide or polypeptide (e.g., a wild-type protein) from which it was derived. For example, a functional derivative of human NeuroD1 contains one or more modifications in its amino acid sequence with respect to the sequence shown in SEQ ID NO: 1. For example, in some embodiments, a functional derivative of human NeuroD1 comprises the amino acid sequence set forth in SEQ ID NO: 4.

[0087] The term “functional derivative” refers to a derivative that retains one or more functions or activities of the naturally occurring or starting peptide or polypeptide (e.g.

a wild-type protein) from which it is derived. For example, in some embodiments, a functional derivative of a reprogramming protein factor as described herein (e.g., NeuroD1) may retain the activity of producing a neuronal phenotype in a glial cell after being expressed in a sufficient amount by the glial cell. In some embodiments, a functional derivative of a reprogramming protein factor may retain the activity of the reprogramming protein factor in reprogramming the glial cell to trans-differentiate into a neuron after being expressed in a sufficient amount by the glial cell. In some embodiments, a functional derivative of a peptide or polypeptide described herein shares at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with respect to the starting (e.g., wild-type) peptide or polypeptide.

[0088] A derivative of polypeptide can be prepared using methods well-known in the art, e.g., by modifying the corresponding nucleic acid molecules encoding the derivative. For example, derivatives may be a substitution, deletion, or insertion of one or more codons encoding the polypeptide that results in a change in the amino acid sequence as compared with the wild-type sequence of the polypeptide. The derivatives can be made using methods well-known in the art such as DNA synthesis, oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis (see, e.g., Carter, 1986, *Biochem J.* 237: 1-7; and Zoller et al., 1982, *Nucl. Acids Res.* 10: 6487-500), cassette mutagenesis (see, e.g., Wells et al., 1985, *Gene* 34: 315-23), or other known techniques can be performed on the cloned DNA to produce the derivatives DNA.

[0089] Those skilled in the art can determine the site (s) in an amino acid sequence of a given protein, where a modification (s) can be made in order to produce functional derivatives. In some embodiments, a functional derivative of a polypeptide comprises one or more modifications to one or more predicted non-essential amino acid residues in its sequence. In some embodiments, modifications made to non-essential amino acid residues can be a conservative substitution as described herein. In some embodiments, modifications made to non-essential amino acid residues can be a substantial substitution described herein. In some embodiments, modifications made to non-essential amino acid residues can be a deletion of the non-essential amino acid residue. In alternative embodiments, one or more modifications can be made to one or more predicted essential amino acid residues in its sequence. In particularly embodiments, the modifications made to essential amino acid residues in a protein sequence can be a conservative substitution as described herein. Methods well-known in the art can be used to analyze a protein (e.g., NeuroD1) sequence to identify essential and non-

essential amino acid residues of the protein. For example, in some embodiments, an amino acid residue of a protein that is not conserved among orthologous gene products is predicted to be a non-essential amino acid residue, while another amino acid residue that is conserved among orthologous gene products is predicted to be an essential amino acid residue. For example, an alignment of twelve NeuroD1 orthologs is shown in Figure 2B, and the conserved residues and non-conserved residues are marked with different shades, respectively.

[0090] In some embodiments, after making one or more modifications to the sequence of a polypeptide (e.g., by making insertions, deletions, or substitutions of amino acids in the original amino acid sequence either systematically, randomly, or at selected sites), functional derivatives of the polypeptide can be identified by testing the resulting derivatives for activity exhibited by the original sequence. For example, to identify functional derivative of a reprogramming protein factor (e.g., NeuroD1) as described herein, nucleic acid molecules encoding the derivative polypeptides can be delivered into a population of starting glial cells under a suitable condition to be expressed at a sufficient level, and assays can be conducted to detect and/or measure one or more neuronal phenotypes in the population of cells and compared the level at which the neuronal phenotype of interest is demonstrated by the population of cells to a control group of glial cells that express the original, unmodified (e.g., wild-type) reprogramming protein factor, and those derivatives that induce the neuronal phenotype in the testing cell population at a comparable level to that of the control population can be selected as functional derivatives. Alternatively, the comparison can be made to a control group of glial cells that do not express the reprogramming protein factor (e.g. transduced with a blank vector), and those derivative that induce the neuronal phenotype in the testing cell population at a greater level than that of the control population can be selected as functional derivatives.

[0091] The term “sequence identity” refers to a relationship between the sequences of two or more biological molecules (e.g., a pair of polynucleotides or multiple polypeptides), as determined by aligning and comparing the respective sequences. “Percent (%) amino acid sequence identity” with respect to a reference amino acid sequence (e.g., a reference polypeptide) is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference amino acid sequence, after aligning the two sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, or MEGALIGN (DNASTar, Inc.) software. Those skilled in the art can determine appropriate parameters for aligning

sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Exemplary parameters for determining relatedness of two or more sequences using the BLAST algorithm, for example, can be as set forth below. Briefly, amino acid sequence alignments can be performed using BLASTP version 2.0.8 (Jan-05-1999) and the following parameters: Matrix: 0 BLOSUM62; gap open: 11; gap extension: 1; x_dropoff: 50; expect: 10.0; wordsize: 3; filter: on. Nucleic acid sequence alignments can be performed using BLASTN version 2.0.6 (Sept-16-1998) and the following parameters: Match: 1; mismatch: -2; gap open: 5; gap extension: 2; x_dropoff: 50; expect: 10.0; wordsize: 11; filter: off. Those skilled in the art will know what modifications can be made to the above parameters to either increase or decrease the stringency of the comparison, for example, and determine the relatedness of two or more sequences.

[0092] The term “vector” refers to a substance that is used to carry or include a nucleic acid sequence, including for example, a nucleic acid sequence encoding a peptide or protein as described herein, in order to introduce a nucleic acid sequence into a host cell, or serve as a transcription template to carry out in vitro transcription reaction in a cell-free system to produce mRNA. Vectors applicable for use include, for example, expression vectors, plasmids, phage vectors, viral vectors, episomes, and artificial chromosomes, which can include selection sequences or markers operable for stable integration into a host cell’s chromosome. Additionally, the vectors can include one or more selectable marker genes and appropriate transcription or translation control sequences. Selectable marker genes that can be included, for example, provide resistance to antibiotics or toxins, complement auxotrophic deficiencies, or supply critical nutrients not in the culture media. Transcription or translation control sequences can include constitutive and inducible promoters, transcription enhancers, transcription terminators, and the like, which are well known in the art. When two or more nucleic acid molecules are to be co-transcribed or co-translated (e.g., nucleic acid molecules encoding two or more different peptides or proteins), both nucleic acid molecules can be inserted, for example, into a single expression vector or in separate expression vectors. For single vector transcription and/or translation, the encoding nucleic acids can be operationally linked to one common transcription or translation control sequence or linked to different transcription or translation control sequences, such as one inducible promoter and one constitutive promoter. The introduction of nucleic acid molecules into a host cell can be confirmed using methods well known in the art. Such methods include, for example, nucleic acid analysis such as Northern blots or polymerase chain reaction (PCR) amplification of mRNA, immunoblotting for expression of gene products, or other suitable analytical methods to test the expression of an introduced nucleic acid sequence or its corresponding gene product. It is understood by those skilled in the

art that the nucleic acid molecules are expressed in a sufficient amount to produce a desired product (e.g., a mRNA transcript of the nucleic acid as described herein) , and it is further understood that expression levels can be optimized to obtain sufficient expression using methods well known in the art.

[0093] The term “administer” or “administration” refers to the act of injecting or otherwise physically delivering a substance as it exists outside the body (e.g., a recombinant AAV as described herein) into a patient, such as by intracranial, mucosal, intradermal, intravenous, intramuscular delivery, and/or any other method of physical delivery described herein or known in the art. When a disease, disorder, condition, or a symptom thereof, is being treated, administration of the substance typically occurs after the onset of the disease, disorder, condition, or symptoms thereof. When a disease, disorder, condition, or symptoms thereof, are being prevented, administration of the substance typically occurs before the onset of the disease, disorder, condition, or symptoms thereof.

[0094] An “effective amount” is generally an amount sufficient to produce a desirable outcome, such as, producing one or more neuronal phenotypes in a population of cells, or in the context of disease management, to reduce the severity and/or frequency of symptoms, eliminate the symptoms and/or underlying cause, prevent the occurrence of symptoms and/or their underlying cause, and/or improve or remediate the damage that results from or is associated with a disease, disorder, or condition, including, for example, infection and neoplasia.

[0095] The terms “subject” and “patient” may be used interchangeably. As used herein, in certain embodiments, a subject is a mammal, such as a non-primate (e.g., cow, pig, horse, cat, dog, rat, etc.) or a primate (e.g., monkey and human) . In specific embodiments, the subject is a human. In one embodiment, the subject is a mammal (e.g., a human) having an infectious disease or neoplastic disease. In another embodiment, the subject is a mammal (e.g., a human) at risk of developing an infectious disease or neoplastic disease.

[0096] The term “phenotype” refers to well-known detectable characteristics of the cells referred to herein. The neuronal phenotype can be, but is not limited to, one or more of: neuronal morphology, expression of one or more neuronal markers, electrophysiological characteristics of neurons, synapse formation and release of neurotransmitter. For example, neuronal phenotype encompasses but is not limited to: characteristic morphological aspects of a neuron such as presence of dendrites, an axon and dendritic spines; characteristic neuronal protein expression and distribution, such as presence of synaptic proteins in synaptic puncta, presence of MAP2 in dendrites; and characteristic electrophysiological signs such as spontaneous and evoked synaptic events. Phenotypes that distinguish a neuron from a non-neuron cell (e.g., a glial cell) as well

as method for detecting and measuring such phenotypes are known to those of ordinary skill in the art.

[0097] The term “detectable probe” refers to a composition that provides a detectable signal. The term includes, without limitation, any fluorophore, chromophore, radiolabel, enzyme, antibody or antibody fragment, and the like, that provide a detectable signal via its activity.

[0098] As used herein, and unless otherwise specified, the term “optional” or “optionally” (e.g., optionally substituted) means that the subsequently described event of circumstances may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, “optionally wherein” means that the features following the wherein may or may not be present and that the description includes either situation when such features are present or absent.

[0099] The term “composition” is intended to encompass a product containing the specified ingredients (e.g., a recombinant AAV) in, optionally, the specified amounts.

[0100] “Substantially all” refers to at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or about 100%.

[0101] As used herein, and unless otherwise indicated, the term “about” or “approximately” means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined. In certain embodiments, the term “about” or “approximately” means within 1, 2, 3, or 4 standard deviations. In certain embodiments, the term “about” or “approximately” means within 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.05%, or less of a given value or range. As used herein, when “about” is used in connection with a numerical range, the term “about” is meant to apply to both ends of such modified range (e.g., “about 5 to 10” means “about 5 to about 10”).

[0102] The singular terms “a,” “an,” and “the” as used herein include the plural reference unless the context clearly indicates otherwise.

[0103] All publications, patent applications, accession numbers, and other references cited in this specification are herein incorporated by reference in their entirety as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided can be different from the actual publication dates which can need to be independently confirmed.

[0104] A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, the descriptions in the Experimental section and examples are intended to illustrate but not limit the scope of invention described in the claims.

5.3 NeuroD1 Expression Cassette

[0105] In one aspect, provided herein are functional nucleic acid molecules for treating glioma. In some embodiments, the functional nucleic acid comprises an expression cassette encoding a NeuroD1 polypeptide, which upon contacting with the glioma cell, is expressed by the glioma cell to produce the encoded NeuroD1 polypeptide. In some embodiment, the expression cassette comprises at least one coding region encoding a NeuroD1 polypeptide (e.g., an open reading frame (ORF)). In some embodiment, the expression cassette further comprises at least one untranslated region (UTR) . In some embodiments, the UTR comprises one or more regulatory elements as described herein. In some embodiments, the expression cassette can comprise any coding sequences as described in this Section 5.3.1 (Coding Region) . In some embodiments, the expression cassette can comprise any regulatory elements described in Section 5.3.2 (Untranslated Regions (UTRs)) .

[0106] In some embodiments, the NeuroD1 expression cassette is part of a single-stranded nucleic acid molecule, including a single-stranded self-complementary DNA molecule. In some embodiments, the single-stranded self-complementary DNA molecule is an artificial AAV genome that can be packaged into a recombinant AAV capsid.

[0107] In other embodiments, the NeuroD1 expression cassette is part of a nucleic acid molecule that is configured to produce a linear nucleic acid molecule, including a single-stranded self-complementary DNA molecule. In some embodiments, the NeuroD1 expression cassette is part of a vector (e.g., a plasmid) that can be processed into a linear recombinant AAV genome in a host cell in the presence of sufficient adenovirus helper functions to permit replication and packaging of the linear recombinant AAV genome by the AAV capsid proteins.

5.3.1 Coding Region

[0108] In some embodiments, the NeuroD1 expression cassette of the present disclosure comprises at least one coding region. In some embodiments, the coding region is an open reading frame (ORF) that encodes for a NeuroD1 polypeptide. In some embodiments, the coding region comprises at least two ORFs, each encoding a NeuroD1 polypeptide. In those embodiments where the coding region comprises more than one ORFs, the encoded NeuroD1 polypeptides can be the same as or different from each other. In some embodiments, the multiple ORFs in a coding region are separated by non-coding sequences.

[0109] In specific embodiments, the coding sequences or amino acid sequences of NeuroD1 polypeptides can be any NeuroD1 polypeptide as described herein. Table 5.3.1 shows exemplary NeuroD1 polypeptides and encoding nucleic acid sequences thereof.

Table 5.3.1 Exemplary NeuroD1 polypeptide and encoding nucleic acid sequences.

SEQUENCE NAME (SEQ ID NO:)	AMINO ACID OR NUCLEIC ACID SEQUENCE
NeuroD1 amino acid sequence v.1 (wild-type) (SEQ ID NO:1)	MTKSYSESGLMGEPQPQGPSSWTDECLSSQDEEHEADKKEDDLETMNA EEDSLRNGGEEDEDEDLEEEEEEEEDDDQKPKRRGPKKKKMTKARLE RFKLRRMKANARERNRMHGLNAALDNLRKVVPCYSKTQKLSKIETLRL AKNYIWALSEILRSQKSPDLVSFVQTLCKGLSQPTTNLVAGCLQLNPRTF LPEQNQDMPPHLPTASASFPVHPYSYQSPGLSPPPYGTMDSSHVHFVKPP PHAYSAALEPFFESPLTDCTSPSFDGPLSPPLSINGNFSFKHEPSAEFEKNY AFTMHYPAATLAGAQSHGSIFSHTAAPRCEIPIDNIMSFDSHSHHERVMS AQLNAIFHD
NeuroD1 coding DNA sequence v.1 (SEQ ID NO:2)	ATGACCAAATCGTACAGCGAGAGTGGGCTGATGGGCGAGCCTCAGCC CCAAGGTCCTCCAAGCTGGACAGACGAGTGTCTCAGTTCTCAGGACG AGGAGCACGAGGCAGACAAGAAGGAGGACGACCTCGAAACCATGAA CGCAGAGGAGGACTCACTGAGGAACGGGGGAGAGGAGGAGGACGA AGATGAGGACCTGGAAGAGGAGGAAGAAGAGGAAGAGGAGGATGA CGATCAAAAAGCCCAAGAGACGCGGCCCAAAAAGAAGAAGATGACT AAGGCTCGCCTGGAGCGTTTTAAATTGAGACGCATGAAGGCTAACGC CCGGGAGCGGAACCGCATGCACGGAAGTGAACGCGCGCTAGACAAC CTGCGCAAGGTGGTGCCTTGCTATTCTAAGACGCAGAAGCTGTCCAA AATCGAGACTCTGCGCTTGGCCAAGAACTACATCTGGGCTCTGTGCG AGA1CCTGCGCTCAGGCAAAAAGCCAGACCTGGTCTCCTTCGTTCAG ACGCTTTGCAAGGGCTTATCCCAACCCACCACCAACCTGGTTGCGGG CTGCCTGCAACTCAATCCTCGGACTTTTCTGCCTGAGCAGAACCAGGA CATGCCCCCCCACCTGCCGACGGCCAGCGCTTCTTCCCTGTACACCC CTACTCCTACCAAGTCCGCTGGGCTGCCAGTCCGCTTACGGTACCAT GGACAGCTCCCATGTCTTCCACGTTAAGCCTCCGCCGCACGCCTACAG CGCAGCGCTGGAGCCCTTCTTTGAAAGCCCTCTGACTGATTGCACCAG CCCTTCTTTGATGGACCCCTCAGCCCGCCGCTCAGCATCAATGGCAA CTTCTCTTTCAAACACGAACCGTCCGCCGAGTTTGAGAAAAATTATGC CTTTACCATGCACTATCCTGCAGCGACACTGGCAGGGGCCCAAGCC ACGGATCAATCTTCTCAGGCACCGCTGCCCTCGCTGCGAGATCCCCA TAGACAATATTATGTCTTCGATAGCCATTACATCATGAGCGAGTCA TGAGTGCCCGAGCTCAATGCCATATTTTCATGATTAA
NeuroD1 coding DNA sequence v.2 Codon Optimized Variant (SEQ ID NO:3)	ATGACCAAAGTCTTACAGCGAGTCCGGCCTGATGGGAGAGCCACAGCC ACAGGGTCCCCCTCCTGGACAGACGAGTGCCTGAGTTCACAGGATG AGGAGCACGAGGCAGACAAGAAGGAGGACGATCTGGAGACCATGAA TGCCGAGGAGGACTCCCTGCGCAACGGCGGGGAGGAGGAGGACGAG GATGAGGACCTGGAGGAAGAGGAGGAGGAGGAGGAGGAGGAGGACGAC GATCAGAAACCCAAGCGTCCGGGCCCCAAGAAGAAGAAGATGACCA AGGCTCGCCTGGAGCGGTTCAAGCTGCGGCGCATGAAGGCCAATGCC CGCGAGCGCAATCGCATGCACGGCCTGAACGCCGCACTTGATAACCT GCGCAAGGTGGTGCCTTGCTACTCCAAGACTCAGAAGCTGAGTAAGA TCGAGACGCTGCGTCTGGCCAAGAATTACATCTGGGCCCTGAGCGAG ATTCTGCGCTCAGGGAAGTCGCCAGATCTGTTGAGCTTCTGTCGAC GCTGTGTAAGGGGCTGTCCAGCCCACCACCAACCTGGTGGCCGGCT

	GCCTCCAGCTCAATCCTCGGACCTTCTGCCCAGCAGAACCAGGAC ATGCCTCCCCACCTGCCTACGGCAAGTGCCAGCTTCCCTGTGCACCT TATTCTTACCAGTCCCCCGGGCTGCCAAGCCCTCCTTACGGAACCATG GACTCCAGCCATGTGTTCACGTGAAGCCTCCTCCTCACGCCCTACAGC GCGGCTCTGGAGCCCTTCTTCGAGAGCCCGCTGACGGACTGCACCAG CCCCAGCTTCGACGGTCTCTGAGCCCACCTCTGAGTATCAACGGAA ACTTCAGCTTCAAGCATGAGCCTTCCGCGGAGTTCGAGAAGAATACTAC GCCTTCACGATGCACTACCCCGCCGCCACACTGGCGGGGGCCAGAG TCACGGCAGCATCTTCTCTGGGACCGCAGCCCCCGCTGCGAGATCC CAATCGACAACATCATGAGCTTCGACTCTCATAGCCACCATGAGCGG GTGATGAGCGCCAGCTCAATGCAATCTTCCATGATTGA
NeuroD1 amino acid sequence v.2 (with an extra residue V encoded by an extra codon GTG (SEQ ID NO:4)	MYTKSYSESGLMGEPQPQGPSPSWTDECLSSQDEEHEADKKEDDLETMN AEEDSLRNGGEEDEDEDELEEEEEEEEEEDDDQKPKRRGPKKKKMTKAR LERFKLRRMKANARERNRMHGLNAALDNLKRVVPCYSKTQKLSKIETL RLAKNYIWALSEILRSGKSPDLVSFVQTLCKGLSQPTTNLVAGCLQLNPR TFLPEQNQDMPPHLPASASFVHPYSYQSPGLSPPYGTMDSSHVFHVK PPPHAYSAALEPFFESPLTDCTSPSFDGPLSPPLSINGNFSFKHEPSAEFEK NYAFTMHYPAATLAGAQSHGSIFSHTAAPRCEIPIDNIMSFDSHSHHERV MSAQLNAIFHD
NeuroD1 coding DNA sequence v.3 (with extra CTG to <u>accommodate a</u> <u>Kozak sequence for</u> <u>transcription</u>) (SEQ ID NO:5)	ATG CTG ACCAAAATCGTACAGCGAGAGTGGGCTGATGGGCGAGCCTC AGCCCCAAGGTCTCCAAGCTGGACAGACGAGTGTCTCAGTTCTCAG GACGAGGAGCAGAGGCAGACAAGAAGGAGGACGACCTCGAAACCA TGAACGCAGAGGAGGACTCACTGAGGAACGGGGGAGAGGAGGAGGA CGAAGATGAGGACCTGGAAGAGGAGGAAGAAGAGGAAGAGGAGGA TGACGATCAAAAGCCCAAGAGACGCGCCCCCAAAAAGAAGAAGATG ACTAAGGCTCGCCTGGAGCGTTTTAAATTGAGACGCATGAAGGCTAA CGCCCCGGGAGCGGAACCGCATGCACGGACTGAACGCGGCGCTAGAC AACCTGCGCAAGGTGGTGCCTTGCTATTCTAAGACGCAGAAGCTGTC CAAAATCGAGACTCTGCGCTTGGCCAAGAATACTACATCTGGGCTCTGT CGGAGATCCTGCGCTCAGGCAAAAAGCCCAGACCTGGTCTCCTTCGTT CAGACGCTTTGCAAGGGCTTATCCCAACCCACCACCAACCTGGTTGC GGGCTGCGCTGCAACTCAATCCTCGGACTTTTCTGCTGAGCAGAACCA GGACATGCCCCCCCCACCTGCCGACGGCCAGCGCTTCCTTCCCTGTACA CCCCTACTCCTACCAGTCGCTGGGCTGCCAGTCCGCCTTACGGTAC CATGGACAGCTCCCATGTCTTCCACGTTAAGCCTCCGCCGCACGCCTA CAGCGCAGCGCTGGAGCCCTTCTTTGAAAGCCCTCTGACTGATTGCAC CAGCCCTTCCTTTGATGGACCCCTCAGCCCGCCGCTCAGCATCAATGG CAACTTCTCTTCAAACACGAACCGTCCGCCGAGTTTGAGAAAAATT ATGCCTTTACCATGCACTATCCTGCAGCGACACTGGCAGGGGCCCCAA AGCCACGGATCAATCTTCTCAGGCACCGCTGCCCCCTCGCTGCGAGAT CCCCATAGACAATATTATGTCCTTCGATAGCCATTACATCATGAGCG AGTCATGAGTGCCCAGCTCAATGCCATATTTTCATGATTGA

[0110] In particular embodiments, the NeuroD1 expression cassette encodes a NeuroD1 polypeptide. In some embodiments, the encoded NeuroD1 polypeptide is a wild-type NeuroD1. In some embodiments, the encoded NeuroD1 is human NeuroD1 having the amino acid sequence of SEQ ID NO:1. In some embodiments, the encoded NeuroD1 is a NeuroD1 polypeptide having the amino acid sequence of SEQ ID NO: 4, where an extra V is located at the second residue.

[0111] In alternative embodiments, the encoded NeuroD1 polypeptide is a functional derivative of NeuroD1. In some embodiments, a functional derivative of NeuroD1 shares at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least

about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with respect to the native (e.g., wild-type) NeuroD1 protein from which it derives.

- [0112] In some embodiments, a functional derivative of NeuroD1 comprises one or more modifications to one or more predicted non-essential amino acid residues in the NeuroD1 sequence. Methods well-known in the art can be used to analyze a protein (e.g., NeuroD1) sequence to identify essential and non-essential amino acid residues of the protein. For example, in some embodiments, an amino acid residue of a protein that is not conserved among orthologous gene products is predicted to be a non-essential amino acid residue, while another amino acid residue that is conserved among orthologous gene products is predicted to be an essential amino acid residue. An exemplary alignment of NeuroD1 orthologs is shown in Figure 2B, and the conserved residues and non-conserved residues are marked with different shades, respectively.
- [0113] In specific embodiments, a functional derivative of NeuroD1 comprises one or more conservative amino acid substitutions at one or more predicted non-essential amino acid residues of NeuroD1. In specific embodiments, a functional derivative of NeuroD1 comprises one or more conservative amino acid substitutions at one or more predicted essential amino acid residues of NeuroD1.
- [0114] In some embodiments, a functional derivative of NeuroD1 retains the NeuroD1 function in producing one or more neuronal phenotypes in a glial cell, which neuronal phenotypes include but are not limited to neuronal morphology, expression of one or more neuronal marker, electrophysiologic characteristics of neurons, synapse formation and release of neurotransmitters. Methods disclosed herein (see e.g., Example section) and/or well-known in the art can be used to measure the one or more neuronal phenotypes. In some embodiments, a functional derivative of NeuroD1 retains the NeuroD1 function in reprogramming a glial cell to trans-differentiate into a neuron.
- [0115] In specific embodiments, a functional derivative of NeuroD1 comprises one or more conservative amino acid substitutions at one or more predicted non-essential amino acid residues, and shares at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with respect to a wild-type NeuroD1 protein. In some embodiments, the wild-type NeuroD1 protein from which the functional derivative is derived is a wild-type human NeuroD1 having SEQ ID NO: 1. In some embodiments, the NeuroD1 protein from which the functional derivative is derived is a NeuroD1 polypeptide having SEQ ID NO: 4.

- [0116] In specific embodiments, a functional derivative of NeuroD1 comprises one or more conservative amino acid substitutions at one or more predicted non-essential amino acid residues, and shares at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with respect to the native (e.g., wild-type) NeuroD1 protein from which it derives, and further retains the function in producing one or more neuronal phenotypes in a glial cell when expressed in a sufficient amount by the glial cell. In some embodiments, the wild-type NeuroD1 protein from which the functional derivative is derived is a wild-type human NeuroD1 having SEQ ID NO: 1. In some embodiments, the NeuroD1 protein from which the functional derivative is derived is a NeuroD1 polypeptide having SEQ ID NO: 4.
- [0117] In specific embodiments, a functional derivative of NeuroD1 comprises one or more conservative amino acid substitutions at one or more predicted non-essential amino acid residues, and shares at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with respect to the native (e.g., wild-type) NeuroD1 protein from which it derives, and further retains the function in reprogramming a glial cell to trans-differentiate into a neuron when expressed in a sufficient amount by the glial cell. In some embodiments, the wild-type NeuroD1 protein from which the functional derivative is derived is a wild-type human NeuroD1 having SEQ ID NO: 1. In some embodiments, the NeuroD1 protein from which the functional derivative is derived is a NeuroD1 polypeptide having SEQ ID NO: 4.
- [0118] In specific embodiments, a functional derivative of NeuroD1 comprises one or more conservative amino acid substitutions at one or more predicted essential amino acid residues, and shares at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with respect to the native (e.g., wild-type) NeuroD1 protein from which it derives, and further retains the function in producing one or more neuronal phenotypes in a glial cell when expressed in a sufficient amount by the glial cell. In some embodiments, the wild-type NeuroD1 protein from which the functional derivative is derived is a wild-type human NeuroD1 having SEQ ID NO: 1. In some embodiments, the NeuroD1 protein from which the functional derivative is derived is a NeuroD1 polypeptide having SEQ ID NO: 4.
- [0119] In specific embodiments, a functional derivative of NeuroD1 comprises one or more conservative amino acid substitutions at one or more predicted essential amino acid

residues, and shares at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with respect to the native (e.g., wild-type) NeuroD1 protein from which it derives, and further retains the function in reprogramming a glial cell to trans-differentiate into a neuron when expressed in a sufficient amount by the glial cell. In some embodiments, the wild-type NeuroD1 protein from which the functional derivative is derived is a wild-type human NeuroD1 having SEQ ID NO: 1. In some embodiments, the NeuroD1 protein from which the functional derivative is derived is a NeuroD1 polypeptide having SEQ ID NO: 4.

[0120] In some embodiments, the encoded NeuroD1 polypeptide is encoded by (a) a DNA sequence of SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 5, (b) a codon-optimized variant of (a), or (c) a transcribed RNA sequence of (a) or (b). In some embodiments, the codon-optimized variant shares at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% sequence identity to SEQ ID NO: 2. In some embodiments, the codon-optimized variant shares at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% sequence identity to SEQ ID NO: 3. In some embodiments, the codon-optimized variant shares at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% sequence identity to SEQ ID NO: 5. In some embodiments, the transcribed RNA sequence has the same sequence as the DNA coding sequences except that thymine bases in the DNA sequence are replaced by uracil bases in the RNA sequence. In particular embodiments, the NeuroD1 expression cassette is mono-cistronic and encodes only one NeuroD1 polypeptide as described herein.

[0121] In alternative embodiments, the NeuroD1 expression cassette is multi-cistronic and encodes multiple NeuroD1 polypeptides as described herein. In some embodiments, a multi-cistronic expression sequence encoding at least two NeuroD1 polypeptides further encodes an internal ribosome entry site (IRES) that separate two ORFs. Without being bound by the theory, it is contemplated that an internal ribosome entry sites (IRES) can act as the sole ribosome binding site, or serve as one of multiple ribosome binding sites of an mRNA. An mRNA molecule containing more than one functional ribosome binding site can encode several peptides or proteins that are translated independently by the ribosomes (e.g., multicistronic mRNA). Accordingly, in some embodiments, the nucleic acid molecule of the present disclosure

(e.g., mRNA) comprises one or more internal ribosome entry sites (IRES) . Examples of IRES sequences that can be used in connection with the present disclosure include, without limitation, those from picomaviruses (e.g., FMDV) , pest viruses (CFFV) , polio viruses (PV) , encephalomyocarditis viruses (ECMV) , foot-and-mouth disease viruses (FMDV) , hepatitis C viruses (HCV) , classical swine fever viruses (CSFV) , murine leukemia virus (MLV) , simian immune deficiency viruses (SIV) or cricket paralysis viruses (CrPV) .

[0122] In particular embodiments, the IRES has a sequence of an IRES from Taura syndrome virus, Triatoma virus, Theiler's encephalomyelitis virus, Simian Virus 40, Solenopsis invicta virus 1, Rhopalosiphum padi virus, Reticuloendotheliosis virus, Human poliovirus 1, Plautia stali intestine virus, Kashmir bee virus, Human rhinovirus 2, Homalodisca coagulata virus-1, Human Immunodeficiency Virus type 1, Homalodisca coagulata virus-1, Himetobi P virus, Hepatitis C virus, Hepatitis A virus, Hepatitis GB virus, Foot and mouth disease virus, Human enterovirus 71, Equine rhinitis virus, Ectropis obliqua picorna-like virus, Encephalomyocarditis virus, Drosophila C Virus, Human coxsackievirus B3, Crucifer tobamovirus, Cricket paralysis virus, Bovine viral diarrhea virus 1, Black Queen Cell Virus, Aphid lethal paralysis virus, Avian encephalomyelitis virus, Acute bee paralysis virus, Hibiscus chlorotic ringspot virus, Classical swine fever virus, Human FGF2, Human SFTPA1, Human AML1/RUNX1, Drosophila antennapedia, Human AQP4, Human AT1R, Human BAG-1, Human BCL2, Human BiP, Human c-IAP1, Human c-myc, Human eIF4G, Mouse NDST4L, Human LEF1, Mouse HIF1 alpha, Human n. myc, Mouse Gtx, Human p27kip1, Human PDGF2/c-sis, Human p53, Human Pim-1, Mouse Rbm3, Drosophila reaper, Canine Scamper, Drosophila Ubx, Human UNR, Mouse UtrA, Human VEGF-A, Human XIAP, Drosophila hairless, S. cerevisiae TFIID, S. cerevisiae YAP1, tobacco etch virus, turnip crinkle virus, EMCV-A, EMCV-B, EMCV-Bf, EMCV-Cf, EMCV pEC9, Picobirnavirus, HCV QC64, Human Cosavirus E/D, Human Cosavirus F, Human Cosavirus JMY, Rhinovirus NAT001, HRV14, HRV89, HRVC-02, HRV-A21, Salivirus A SH1, Salivirus FHB, Salivirus NG-J1, Human Parechovirus 1, Crohivirus B, Yc-3, Rosavirus M-7, Shanbavirus A, Pasivirus A, Pasivirus A 2, Echovirus E14, Human Parechovirus 5, Aichi Virus, Hepatitis A Virus HA16, Phopivirus, CVA10, Enterovirus C, Enterovirus D, Enterovirus J, Human Pegivirus 2, GBV-C GT110, GBV-C K1737, GBV-C Iowa, Pegivirus A 1220, Pasivirus A 3, Sapelovirus, Rosavirus B, Bakunsa Virus, Tremovirus A, Swine Pasivirus 1, PLV-CHN, Pasivirus A, Sicinivirus, Hepacivirus K, Hepacivirus A, BVDV1, Border Disease Virus, BVDV2, CSFV-PK15C, SF573 Dicistrovirus, Hubei Picorna-like Virus, CRPV, Apodemus Agrarius Picornavirus, Caprine Kobuvirus, Parabovirus, Salivirus A BN5, Salivirus A BN2, Salivirus A 02394, Salivirus A GUT,

Salivirus A CH, Salivirus A SZ1, Salivirus FHB, CVB3, CVB1, Echovirus 7, CVB5, EVA71, CVA3, CVA12, EV24, or an aptamer to eIF4G.

5.3.2 Untranslated Regions (UTRs)

[0123] In some embodiments, the NeuroD1 expression cassette comprises one or more untranslated regions (UTRs).

[0124] In the particular embodiments, the untranslated region (UTR) located upstream (to the 5' -end) of the coding region is referred to herein as the 5' -UTR, and the UTR located upstream (to the 3' -end) of the coding region is referred to herein as the 3' -UTR. In particular embodiments, the NeuroD1 expression cassette comprises both a 5' -UTR and a 3' -UTR. In some embodiments, the NeuroD1 expression cassette comprises a Kozak sequence (e.g., in the 5' -UTR). In some embodiments, the NeuroD1 expression cassette comprises a polyadenylation signal (e.g., in the 3' -UTR). In particular embodiments, the NeuroD1 expression cassette comprises a polyadenylation signal having the sequence set forth in SEQ ID NO: 9 located in the 3' -UTR. In some embodiments, the NeuroD1 expression cassette comprises stabilizing region (e.g., in the 3' -UTR). In some embodiments, the NeuroD1 expression cassette comprises one or more intronic regions capable of being excised during splicing (e.g., in the 5' -UTR). In one specific embodiment, the NeuroD1 expression cassette comprises a chimeric intronic comprising the sequence set forth in SEQ ID NO: 8 located in the 5' -UTR. In some embodiments, the NeuroD1 expression cassette comprises a promoter (e.g., in the 5' -UTR). In a specific embodiment, the NeuroD1 expression cassette comprises a CMV promoter comprising the sequence set forth in SEQ ID NO:7 located in the 5' -UTR. In some embodiments, the NeuroD1 expression cassette comprises a transcription enhancer element (e.g., in the 5' -UTR or 3' -UTR). In a specific embodiment, the NeuroD1 expression cassette comprises a CMV enhancer comprising the sequence set forth in SEQ ID NO: 6 located in the 5' -UTR. In a specific embodiment, the nucleic acid molecule comprises one or more region selected from a 5' -UTR, and a coding region. In a specific embodiment, the nucleic acid molecule comprises one or more region selected from a coding region and a 3' -UTR. In a specific embodiment, the nucleic acid molecule comprises one or more region selected from a 5' -UTR, a coding region, and a 3' -UTR.

[0125] In some embodiments, the sequence of an UTR can be homologous or heterologous to the sequence of the coding region found in a nucleic acid molecule. Multiple UTRs can be included in a nucleic acid molecule and can be of the same or different sequences, and/or genetic origin. According to the present disclosure, any portion of UTRs in a nucleic acid molecule (including none) can be codon optimized and any may independently contain one or more different structural or chemical modification, before and/or after codon optimization.

- [0126] In some embodiments, a NeuroD1 expression cassette of the present disclosure comprises UTRs and coding regions that are homologous with respect to each other. In other embodiments, a NeuroD1 expression cassette of the present disclosure comprises UTRs and coding regions that are heterologous with respect to each other. In some embodiments, to monitor the activity of a UTR sequence, a nucleic acid molecule comprising the UTR and a coding sequence of a detectable probe can be administered in vitro (e.g., cell or tissue culture) or in vivo (e.g., to a subject), and an effect of the UTR sequence (e.g., modulation on the expression level, cellular localization of the encoded product, or half-life of the encoded product) can be measured using methods known in the art.
- [0127] Combinations of regulatory elements may be used to drive expression of an operably linked coding sequence. According to the present disclosure, homologues and functional variants of ubiquitous or cell type-specific promoters may be used in expressing the operably linked coding sequence as described herein. The terms “promoter homologue” and “promoter variant” refer to a promoter which has substantially similar functional properties to confer the desired type of expression, such as cell type-specific expression of the NeuroD1 polypeptide or ubiquitous expression of the NeuroD1 polypeptide, of an operably linked coding sequence of the NeuroD1 polypeptide compared to a given promoter disclosed herein. For example, a promoter homologue or promoter variant has substantially similar functional properties to confer cell type-specific expression of an operably linked coding sequence encoding the NeuroD1 polypeptide compared to any of a GFAP, Aldh1L1, NG2, lcn2, S100b, Sox9, CAG, CMV, ubiquitin, or EF-1a promoter.
- [0128] One of skill in the art will recognize that one or more nucleic acid mutations can be introduced without altering the functional properties of a given promoter. Mutations can be introduced using standard molecular biology techniques, such as DNA synthesis, site-directed mutagenesis and PCR-mediated mutagenesis, to produce promoter variants. As used herein, the term “promoter variant” refers to either an isolated naturally occurring or a recombinantly prepared variation of a reference promoter, such as, but not limited to GFAP, Aldh1L1, NG2, lcn2, S100b, Sox9, CAG, CMV, ubiquitin, or EF-1a promoter.
- [0129] It is known in the art that promoters from other species are functional, e.g. the mouse Aldh1L1 promoter is known to be functional in human cells. Homologues and homologous promoters from other species can be identified using bioinformatics tools known in the art, see for example, Xuan et al., 2005, *Genome Biol* 6: R72; Zhao et al., 2005, *Nucl Acid Res* 33: D103-107; and Halees et al. 2003, *Nucl. Acids. Res.* 2003 31: 3554-3559.

- [0130] Structurally, homologues and variants of a cell type-specific promoter or an ubiquitous promoter can have at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater, nucleic acid sequence identity to the reference promoter and include a site for binding of RNA polymerase and, optionally, one or more binding sites for transcription factors.
- [0131] In some embodiments, the UTR of a nucleic acid molecule of the present disclosure (e.g., NeuroD1 expression cassette) comprises at least one translation enhancer element (TEE) that functions to increase the amount of polypeptide or protein produced from the nucleic acid molecule. In some embodiments, the TEE is located in the 5' -UTR of the nucleic acid molecule. In other embodiments, the TEE is located at the 3' -UTR of the nucleic acid molecule. In yet other embodiments, at least two TEE are located at the 5' -UTR and 3' -UTR of the nucleic acid molecule respectively. In some embodiments, a nucleic acid molecule of the present disclosure can comprise one or more copies of a TEE sequence or comprise more than one different TEE sequences. In some embodiments, different TEE sequences that are present in a nucleic acid molecule of the present disclosure can be homologues or heterologous with respect to one another.
- [0132] In some embodiments, the TEE sequence is derived from a promoter sequence of a gene. In some embodiments, a promoter can be derived entirely from a single gene. In other embodiments, a promoter can be chimeric, having portions derived from more than one gene.
- [0133] In some embodiments, the TEE sequence used in connection with the present disclosure can drive expression of an operably linked expression sequence preferentially in glial cells. In some embodiments, the TEE sequence drives expression of an operably linked expression sequence preferentially in astrocytes. In some embodiments, the TEE sequence drives expression of an operably linked expression sequence preferentially in reactive astrocytes. In some embodiments, the TEE sequence drives expression of an operably linked expression sequence preferentially in NG2 cells. In some embodiments, the TEE sequence drives expression of an operably linked expression sequence preferentially in reactive NG2 cells. In some embodiments, the TEE sequence drives expression of an operably linked expression sequence preferentially in Müller glia cells.
- [0134] Additionally, various TEE sequences that are known in the art and can be used in connection with the present disclosure. For example, in some embodiments, the TEE can be an internal ribosome entry site (IRES) , HCV-IRES or an IRES element. Chappell et al. Proc. Natl. Acad. Sci. USA 101: 9590-9594, 2004; Zhou et al. Proc. Natl. Acad. Sci. 102: 6273-6278, 2005. Additional internal ribosome entry site (IRES) that can be used in connection with the present disclosure include but are

not limited to those described in U.S. Patent No. 7,468,275, U.S. Patent Publication No. 2007/0048776 and U.S. Patent Publication No. 2011/0124100 and International Patent Publication No. WO2007/025008 and International Patent Publication No. WO2001/055369, the content of each of which is enclosed herein by reference in its entirety. In some embodiments, the TEE can be those described in Supplemental Table 1 and in Supplemental Table 2 of Wellensiek et al Genome-wide profiling of human cap-independent translation-enhancing elements, *Nature Methods*, 2013 Aug; 10 (8) : 747–750; the content of which is incorporated by reference in its entirety.

[0135] Additional exemplary TEEs that can be used in connection with the present disclosure include but are not limited to the TEE sequences disclosed in U.S. Patent No. 6,310,197, U.S. Patent No. 6,849,405, U.S. Patent No. 7,456,273, U.S. Patent No. 7,183,395, U.S. Patent Publication No. 2009/0226470, U.S. Patent Publication No. 2013/0177581, U.S. Patent Publication No. 2007/0048776, U.S. Patent Publication No. 2011/0124100, U.S. Patent Publication No. 2009/0093049, International Patent Publication No. WO2009/075886, International Patent Publication No. WO2012/009644, and International Patent Publication No. WO1999/024595, International Patent Publication No. WO2007/025008, International Patent Publication No. WO2001/055371, European Patent No. 2610341, European Patent No. 2610340, the content of each of which is enclosed herein by reference in its entirety.

[0136] In various embodiments, a nucleic acid molecule of the present disclosure (e.g., an *NeuroD1* expression cassette) comprises at least one UTR that comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55 or more than 60 TEE sequences. In some embodiments, the TEE sequences in the UTR of a nucleic acid molecule are copies of the same TEE sequence. In other embodiments, at least two TEE sequences in the UTR of a nucleic acid molecule are of different TEE sequences. In some embodiments, multiple different TEE sequences are arranged in one or more repeating patterns in the UTR region of a nucleic acid molecule. For illustrating purpose only, a repeating pattern can be, for example, ABABAB, AABBAABBAABB, ABCABCABC, or the like, where in these exemplary patterns, each capitalized letter (A, B, or C) represents a different TEE sequence. In some embodiments, at least two TEE sequences are consecutive with one another (i.e., no spacer sequence in between) in a UTR of a nucleic acid molecule. In other embodiments, at least two TEE sequences are separated by a spacer sequence. In some embodiments, a UTR can comprise a TEE sequence-spacer sequence module that is repeated at least once, at least twice, at least 3 times, at least 4 times, at least 5 times, at

least 6 times, at least 7 times, at least 8 times, at least 9 times, or more than 9 times in the UTR. In any of the embodiments described in this paragraph, the UTR can be a 5' -UTR, a 3' -UTR or both 5' -UTR and 3' -UTR of a nucleic acid molecule.

[0137] In some embodiments, the UTR of a nucleic acid molecule of the present disclosure comprises at least one translation suppressing element that functions to decrease the amount of polypeptide or protein produced from the nucleic acid molecule. In some embodiments, the UTR of the nucleic acid molecule comprises one or more miR sequences or fragment thereof (e.g., miR seed sequences) that are recognized by one or more microRNA. In some embodiments, the UTR of the nucleic acid molecule comprises one or more stem-loop structure that downregulates translational activity of the nucleic acid molecule. Other mechanisms for suppressing translational activities associated with nucleic acid molecules are known in the art. In some of the embodiments described in this paragraph, the nucleic acid molecule is linear, and the UTR can be a 5' -UTR, a 3' -UTR or both 5' -UTR and 3' -UTR of a nucleic acid molecule.

[0138] Table 5.3.2 shows exemplary 5' -UTR and 3' -UTR sequences that can be operably linked to a NeuroD1 coding sequence as described herein.

Table 5.3.2 Examples of UTRs.

SEQUENCE NAME (SEQ ID NO:)	NUCLEIC ACID SEQUENCE
5' UTR CMV Enhancer (SEQ ID NO:6)	GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCA TAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAA ATGGCCCCGCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCA ATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGA CGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACA TCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG GTAAATGGCCCCGCTGGCATTTATGCCAGTACATGACCTTATGGGAC TTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATG
5' UTR CMV Promoter (SEQ ID NO:7)	GTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGA CTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTT GTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACT CCGCCCCATTGACGCAATGGGCGGTAGGCGTGTACGGTGGGAGGTC TATATAAGCAGAGCT
5' UTR Chimeric Intron (SEQ ID NO:8)	GTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACT GGGCTTGTGAGACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTA TTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAG
3' UTR poly- adenylation signal (SEQ ID NO:9)	CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTGCC TTCCTTGACCCCTGGAAGGTGCCACTCCCACTGTCTTTCTTAATAAAA TGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGG GGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGAGAAT AGCAGGCATGCTGGGGA
5' UTR (SEQ ID NO:10)	GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCA TAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAA ATGGCCCCGCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCA ATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGA CGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACA TCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG GTAAATGGCCCCGCTGGCATTTATGCCAGTACATGACCTTATGGGAC TTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATG GTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGA CTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTT GTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACT CCGCCCCATTGACGCAATGGGCGGTAGGCGTGTACGGTGGGAGGTC TATATAAGCAGAGCTGTAAGTATCAAGGTTACAAGACAGGTTTAAGG
	AGACCAATAGAAACTGGGCTTGTGAGACAGAGAAGACTCTTGCGTT TCTGATAGGCACCTATTGGTCTTACTGACATCCACTTTGCCTTTCTCTC CACAG
3' UTR (SEQ ID NO:11)	CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTGCC TTCCTTGACCCCTGGAAGGTGCCACTCCCACTGTCTTTCTTAATAAAA TGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGG GGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGAGAAT AGCAGGCATGCTGGGGA

[0139] In some embodiments, the expression cassette is mono-cistronic and encodes one copy of a NeuroD1 polypeptide. In some embodiments, the encoded NeuroD1 polypeptide can be any NeuroD1 polypeptide as described in Section 5.3.1 (Coding Region) . In some embodiments, the encoded NeuroD1 polypeptide comprises an amino acid sequence having at least 95% sequence identity to the sequence set forth in SEQ ID NO: 1. In some embodiments, the encoded NeuroD1 polypeptide comprises

an amino acid sequence set forth in SEQ ID NO: 4. In some embodiments, coding sequence that encodes the NeuroD1 polypeptide comprises the nucleic acid sequence as set forth in SEQ ID NO: 2 or a codon-optimized variant thereof. In some embodiments, coding sequence that encodes the NeuroD1 polypeptide comprises the nucleic acid sequence as set forth in SEQ ID NO: 3 or a codon-optimized variant thereof. In some embodiments, coding sequence that encodes the NeuroD1 polypeptide comprises the nucleic acid sequence as set forth in SEQ ID NO: 5 or a codon-optimized variant thereof. In some embodiments, coding sequence that encodes the NeuroD1 polypeptide consists of the nucleic acid sequence as set forth in SEQ ID NO: 5.

[0140] In some embodiments, the expression cassette further comprises one or more untranslated regions (UTRs) . In some embodiments, the UTR comprises one or more regulatory elements operably linked to the coding sequence that encodes the NeuroD1 polypeptide. In some embodiments, the UTRs can be any UTR as described in Section 5.3.2 (Untranslated Regions (UTRs)) . In some embodiments, the expression cassette comprises a 5' UTR located upstream (to the 5' end) of the coding sequence that encodes the NeuroD1 polypeptide. In some embodiments, the 5' -UTR comprises, from the 5' to 3' direction, a CMV enhancer, a CMV promoter, and a chimeric intron.

[0141] In some embodiments, the CMV enhancer comprises the sequence set forth in SEQ ID NO: 6, or a functional variant having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereof. In some embodiments, the CMV enhancer consists of the sequence set forth in SEQ ID NO: 6.

[0142] In some embodiments, the CMV promoter comprises the sequence set forth in SEQ ID NO: 7, or a functional variant having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereof. In some embodiments, the CMV promoter consists of the sequence set forth in SEQ ID NO: 7.

[0143] In some embodiments, the chimeric intron comprises the sequence set forth in SEQ ID NO: 8, or a functional variant having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereof. In some embodiments, the chimeric intron consists of the sequence set forth in SEQ ID NO: 8.

[0144] In some embodiments, the 5' UTR of the expression cassette comprises the sequence set forth in SEQ ID NO: 10. In some embodiments, the expression cassette comprises a 5' UTR comprising the sequence set forth in SEQ ID NO: 10, wherein the 5' UTR is linked to the 5' end of a coding sequence that encodes a NeuroD1 polypeptide comprising an amino acid sequence having at least 95% sequence identity to the

sequence set forth in SEQ ID NO: 1. In some embodiments, the expression cassette comprises a 5' UTR comprising the sequence set forth in SEQ ID NO: 10, wherein the 5' UTR is linked to the 5' end of a coding sequence that encodes a NeuroD1 polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 4. In some embodiments, the expression cassette comprises a 5' UTR comprising the sequence set forth in SEQ ID NO: 10, wherein the 5' UTR is linked to the 5' end of a coding sequence that encodes a NeuroD1 polypeptide comprising the nucleic acid sequence set forth in SEQ ID NO: 5, or a codon optimized variant thereof. In some embodiments, the expression cassette comprises a 5' UTR comprising the sequence set forth in SEQ ID NO: 10, wherein the 5' UTR is linked to the 5' end of a coding sequence that encodes a NeuroD1 polypeptide comprising the nucleic acid sequence set forth in SEQ ID NO: 5.

[0145] In some embodiments, the expression cassette comprises a 3' UTR located downstream (to the 3' end) of the coding sequence that encodes the NeuroD1 polypeptide. In some embodiments, the 3' -UTR comprises a polyadenylation signal comprising the sequence set forth in SEQ ID NO: 9, or a functional variant having at least 90% sequence identity thereof. In some embodiments, the polyadenylation signal consists of the sequence set forth in SEQ ID NO: 9. In some embodiments, the polyadenylation signal comprises a SV40 polyadenylation signal. In some embodiments, the polyadenylation signal comprises a human beta globin polyadenylation signal. In some embodiments, the polyadenylation signal comprises a polyadenylation signal originated from a human gene. In some embodiments, the polyadenylation signal comprises a polyadenylation signal originated from a non-human gene.

[0146] In some embodiments, the 3' UTR of the expression cassette comprises the sequence set forth in SEQ ID NO: 11. In some embodiments, the expression cassette comprises a 3' UTR comprising the sequence set forth in SEQ ID NO: 11, wherein the 3' UTR is linked to the 3' end of a coding sequence that encodes a NeuroD1 polypeptide comprising an amino acid sequence having at least 95% sequence identity to the sequence set forth in SEQ ID NO: 1. In some embodiments, the expression cassette comprises a 3' UTR comprising the sequence set forth in SEQ ID NO: 11, wherein the 3' UTR is linked to the 3' end of a coding sequence that encodes a NeuroD1 polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 4. In some embodiments, the expression cassette comprises a 3' UTR comprising the sequence set forth in SEQ ID NO: 11, wherein the 3' UTR is linked to the 3' end of a coding sequence that encodes a NeuroD1 polypeptide comprising the nucleic acid sequence set forth in SEQ ID NO: 5, or a codon optimized variant thereof. In some embodiments, the expression cassette comprises a 3' UTR comprising the sequence set forth in SEQ ID NO: 11, wherein the 3' UTR is linked to the 3' end of a coding

sequence that encodes a NeuroD1 polypeptide comprising the nucleic acid sequence set forth in SEQ ID NO: 5.

[0147] Table 5.3 below lists the sequences of functional fragments in a NeuroD1 expression cassette according to some embodiments described herein. In some embodiments, the expression cassette comprises, from the 5' to 3' direction, a CMV enhancer (SEQ ID NO: 6) , a CMV promoter (SEQ ID NO:7) , a chimeric intron (SEQ ID NO: 8) , a coding sequence (SEQ ID NO: 5) that encodes a NeuroD1 polypeptide (SEQ ID NO: 4) , and a polyadenylation signal (SEQ ID NO: 9) . In specific embodiments, the CMV enhancer (SEQ ID NO: 6) , CMV promoter (SEQ ID NO: 7) , chimeric intron (SEQ ID NO: 8) , coding sequence that encodes a NeuroD1 polypeptide (SEQ ID NO: 5) , and polyadenylation signal (SEQ ID NO:9) are connected directly to each other in the 5' to 3' order, and the expression cassette comprises the sequence of SEQ ID NO: 12.

Table 5.3 Example of a NeuroD1 Expression Cassette

NeuroD1 Expression Cassette	
CMV Enhancer (SEQ ID NO:6)	GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACG GGGTCAATTAGTTCA TAGCCCATATATGGAGTTCCGCGTTA CATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAA CGACCCCGCCCA TGGACGTCAATAATGACGTATGTTCCT ATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGG TGGACTATTTACGGTAAACTGCCCCACTTGGCAGTACAICA AGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAAT GACGGTAAATGGCCCGCTGGCATTATGCCCAGTACATGA CCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTA GTCAICGCTATTACCA TG
CMV Promoter (SEQ ID NO:7)	GTGATGCGGTTTTTGGCAGTACATCAATGGGCGTGGATAGC GGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTTGA CGTCAATGGGAGTTTGT TTTGGCACCAAAATCAACGGGAC TTTCCAAAATGTCTGTAACAACTCCGCCCCATTGACGCAAA TGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAG AGCT
Chimeric intron (SEQ ID NO:8)	GTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAAT AGAAACTGGGCTTGTCTGAGACAGAGAAGACTCTTGCGTTT CTGATAGGCACCTATTGGTCTTACTGACATCCACTTTGCC TTCTCTCCACAG
NeuroD1 coding sequence (with extra <u>GTG</u>) (SEQ ID NO:5)	ATG <u>GTG</u> ACCAAAATCGTACAGCGAGAGTGGGCTGATGGGC GAGCCTCAGCCCCAAGGTCCTCCAAGCTGGACAGACGAGT GTCTCAGTTCTCAGGACGAGGAGCACGAGGCAGACAAGA AGGAGGACGACCTCGAAAACCATGAACGCAGAGGAGGACT CACTGAGGAACGGGGGAGAGGAGGAGGACGAAGATGAG GACCTGGAAGAGGAGGAAGAAGAGGAAGAGGAGGATGA CGATCAAAAGCCCAAGAGACGCGGCCCAAAAAGAAGAA GATGACTAAAGGCTCGCCTGGAGCGTTTTTAAATTGAGACGC ATGAAGGCTAACGCCCCGGGAGCGGAACCGCATGCACGGA CTGAACGCGGCGCTAGACAACCTGCGCAAGGTGGTGCCTT CCTATTCTAAGACCGCAGAAAGCTGTCCAAAATCGAGACTCT GCGCTTGGCCAAAGAACTACATCTGGGCTCTGTGCGGAGATC CTGCGCTCAGGCAAAAGCCAGACCTGGTCTCCTTCGTTT AGACGCTTTGCAAGGGCTTATCCCAACCCACCACCAACCT GGTTGCGGGCTGCCTGCAACTCAATCCTCGGACTTTTCTGC CTGAGCAGAACCCAGGACATGCCCCCCCACCTGCCGACGGC CAGCGCTTCTTCCCTGTACACCCCTACTCCTACCAGTCCG CTGGGCTGCCCAGTCCGCTTACGGTACCATGGACAGCTC CCATGTCTTCCACGTTAAGCC TCCGCCGCACGCCTACAGC GCAGCGCTGGAGCCCTTCTTTGAAAAGCCCTCTGACTGATT GCACCAGCCCTTCTTTGATGGACCCCTCAGCCCCGCGCTC AGCATCAATGGCAACTTCTCTTTCAAACACGAACCGTCCG CCGAGTTTGAGAAAAATTATGCCTTTACCATGCACTATCCT GCAGCGACACTGGCAGGGGGCCCAAGGCCACGGATCAATC

	TTCTCAGGCACCGCTGCCCCCTCGCTGCGAGATCCCCATAG ACAAATATTATGTCTTCGATAGCCATTCACATCATGAGCG AGTCATGAGTGCCCAGCTCAATGCCATATTTTCATGATTGA
Encoded NeuroD1 amino acid sequence (with an extra residue <u>Y</u>) (SEQ ID NO:4)	MYTKSYSESLMGEPQPQPPSWTDECLSSQDEEHEADKKE DDLETMNAEEDSLRNGGEEDEDEDEEEEEEEEEEDDDQKP KRRGPKKKKMTKARLERFKLRRMKANARERNRMHGLNAA LDNLRKVVPYCYSKTQKLSKIETLRLAKNYIWALSEILRSGKSP DLVSFVQTLCKGLSQPTTNLVAGCLQLNPRTFLPEQNQDMPP HLPTASASFPVHPYSYQSPGLSPPPYGTMDSSHVFHVKPPPHA YSAALEPFFESPLTDCTSPSFDGPLSPPLSINGNPSFKHEPSAEF EKNYAFTMHYPAAATLAGAQSHGSIFSGTAAPRCEIPIDNIMSF DSHSHHERVMSAQLNAIFHD
Polyadenylation signal (SEQ ID NO:9)	CTGTGCCTTCTAGTTGCCAGCCATCTGTGTGTTTGCCCTCC CCCGTGCCCTTCTTGACCCCTGGAAGGTGCCACTCCCCTGT CCTTTCTAATAAAATGAGGAAATTGCATCGCATTGTCTG AGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGG ACAGCAAGGGGGAGGATTGGGAAGAGAATAGCAGGCATG CTGGGGA
Full-Length NeuroD1 Expression Cassette comprising SEQ ID NOS: 6, 7, 8, 5, and 9 (SEQ ID NO:12)	GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACG GGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTA CATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAA CGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCC ATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGG TGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCA AGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAAT GACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGA CCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTA GTCATCGCTATTACCATGGTGATGCGGTTTGGCAGTACAT CAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCA AGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCA CCAAAATCAACGGGACTTTCACAAAATGTCGTAACAACCTC GCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGG AGGTCTATATAAGCAGAGCTGTAAGTATCAAGGTTACAAG ACAGGTTTAAGGAGACCAATAGAAACTGGGCTTGTGCGAG ACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGGT CTTACTGACATCCACTTTCCTTTCTCTCCACAGATGGTGA CCAAATCGTACAGCGAGAGTGGGCTGATGGGCGAGCCTC AGCCCCAAGGTCCTCCAAGCTGGACAGACGAGTGTCTCAG TTCTCAGGACGAGGAGCAGAGGCAGACAAGAAGGAGGA CGACCTCGAAACCATGAACGCAGAGGAGGACTCACTGAG GAACGGGGGAGAGGAGGAGGACGAAGATGAGGACCTGG AAGAGGAGGAAGAAGAGGAAGAGGAGGATGACGATCAA AAGCCCAAGAGACGCGGCCCCAAAAAGAAGAAGATGACT AAGGCTCGCCTGGAGCGTTTTAAATTGAGACGCATGAAGG CTAACGCCCCGGGAGCGGAACCGCATGCACGGACTGAACG CGGCGCTAGACAACCTGCGCAAGGTGGTGCCTTGCTATT TAAGACGCAGAAGCTGTCCAAAATCGAGACTCTGCGCTTG GCCAAGAATACATCTGGGCTCTGTCGGAGATCCTGCGCT

	CAGGCAAAAGCCCAGACCTGGTCTCCTTCGTTTCAGACGCT TTGCAAGGGCTTATCCCAACCCACCACCAACCTGGTTGCG GGCTGCCTGCAACTCAATCCTCGGACTTTTCTGCCTGAGCA GAACCAGGACATGCCCCCCCACCTGCCGACGGCCAGCGCT TCCTTCCTGTACACCCCTACTCCTACCAGTCGCCTGGGCT GCCCAGTCCGCCTTACGGTACCATGGACAGCTCCCATGTC TTCCACGTTAAGCCTCCGCCGCACGCCTACAGCCGAGCGC TGGAGCCCTTCTTTGAAAGCCCTCTGACTGATTGCACCAG CCCTTCCTTTGATGGACCCCTCAGCCCCCGCTCAGCATCA ATGGCAACTTCTCTTTCAAACACGAACCGTCCGCCGAGTT TGAGAAAAATTATGCCTTTACCATGCACTATCCTGCAGCG ACACTGGCAGGGGGCCAAAGCCACGGATCAATCTTCTCAG GCACCGCTGCCCTCGCTGCGAGATCCCCATAGACAATAT TATGTCCTTCGATAGCCATTACATCATGAGCGAGTCATG AGTGCCAGCTCAATGCCATATTTTCATGATTGACTGTGCC TCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCGTGCC TTCTTTGACCCCTGGAAGGTGCCACTCCCACTGTCTTTCT AATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTG TCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAG GGGGAGGATTGGGAAGAGAATAGCAGGCATGCTGGGGA
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5.4 Self-complimentary AAV (scAAV) Genome

- [0148] In some embodiments, the expression cassette described herein is part of a single-stranded nucleic acid molecule. In some embodiments, the expression cassette is part of a single-stranded self-complementary nucleic acid molecule. In some embodiments, the single-stranded self-complementary nucleic acid molecule is a DNA molecule. In some embodiments, the single-stranded self-complementary nucleic acid molecule is an artificial AAV genome that can be packaged into a recombinant AAV capsid.
- [0149] In some embodiments, the single-stranded self-complementary nucleic acid molecule comprises a pair of terminal reverse complementary fragments each located on one end of the nucleic acid molecule, an expression cassette comprising at least one coding sequence that encodes a NeuroD1 polypeptide, an anti-sense region that is reverse-complementary to the expression cassette, and a central reverse-complementary fragment that connects the expression cassette and anti-sense region, wherein each of the pair of terminal reverse-complementary fragments and the central reverse-complementary fragment is capable of forming a stem-loop structure. In some embodiments, the pair of terminal reverse complementary fragments comprises a 5' terminal complementary fragment and a 3' terminal complementary fragment. In some embodiments, the sequences of the pair of terminal reverse complementary fragments are reverse complementary to one another. In some embodiments, the expression cassette can be any expression cassette as described in Section 5.3 (NeuroD1 Expression Cassette) .
- [0150] In some embodiments, the pair of terminal reverse complementary fragments each comprises a first inverted terminal repeat (ITR) of a first AAV genome. In some

embodiments, the first ITR is selected from the genomic ITR sequences of AAV serotypes AAV1, AAV2, AAV2tYF, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAVrh10, AAV. rh20, AAV. rh39, AAV. Rh74, AAV. RHM4-1, AAV. hu37, AAV. Anc80, AAV. Anc80L65, rAAV. 7m8, AAV. PHP. B, AAV. PHP. eB, AAV2.5, AAV2tYF, AAV3B, AAV. LK03, AAV. HSC1, AAV. HSC2, AAV. HSC3, AAV. HSC4, AAV. HSC5, AAV. HSC6, AAV. HSC7, AAV. HSC8, AAV. HSC9, AAV. HSC10, AAV. HSC11, AAV. HSC12, AAV. HSC13, AAV. HSC14, AAV. HSC15, and AAV. HSC16.

[0151] In some embodiments, the first ITR is selected from the genomic ITR sequences of AAV serotypes 1 to 8. In some embodiments, at least one of the pair of terminal reverse complementary fragments comprises the 5' ITR sequence from the AAV1 genome. In some embodiments, at least one of the pair of terminal reverse complementary fragments comprises the 5' ITR sequence from the AAV2 genome. In some embodiments, at least one of the pair of terminal reverse complementary fragments comprises the 5' ITR sequence from the AAV3 genome. In some embodiments, at least one of the pair of terminal reverse complementary fragments comprises the 5' ITR sequence from the AAV4 genome. In some embodiments, at least one of the pair of terminal reverse complementary fragments comprises the 5' ITR sequence from the AAV5 genome. In some embodiments, at least one of the pair of terminal reverse complementary fragments comprises the 5' ITR sequence from the AAV6 genome. In some embodiments, at least one of the pair of terminal reverse complementary fragments comprises the 5' ITR sequence from the AAV7 genome. In some embodiments, at least one of the pair of terminal reverse complementary fragments comprises the 5' ITR sequence from the AAV8 genome. In some embodiments, at least one of the pair of terminal reverse complementary fragments comprises a functional terminal resolution site (trs). In some embodiments, at least one of the pair of terminal reverse complementary fragments comprises the full-length ITR sequence from the AAV genome. In some embodiments, at least one of the pair of terminal reverse complementary fragments each comprises a truncated version of the ITR sequence from the AAV genome. In some embodiments, at least one of the pair of terminal reverse complementary fragments comprises the wild-type ITR sequence from the AAV genome. In some embodiments, at least one of the pair of terminal reverse complementary fragments comprises a mutated ITR sequence from the AAV genome. In some embodiments, the sequences of the pair of terminal reverse complementary fragments are reverse complementary to one another.

[0152] In some embodiments, the central reverse complementary fragment comprises a second inverted terminal repeat (ITR) of a second AAV genome. In some embodiments, the second ITR is selected from the genomic ITR sequences of AAV

serotypes 1 to 8. In some embodiments, the central reverse complementary fragment comprises the 3' ITR sequence from the AAV1 genome. In some embodiments, the central reverse complementary fragment comprises the 3' ITR sequence from the AAV2 genome. In some embodiments, the central reverse complementary fragment comprises the 3' ITR sequence from the AAV3 genome. In some embodiments, the central reverse complementary fragment comprises the 3' ITR sequence from the AAV4 genome. In some embodiments, the central reverse complementary fragment comprises the 3' ITR sequence from the AAV5 genome. In some embodiments, the central reverse complementary fragment comprises the 3' ITR sequence from the AAV6 genome. In some embodiments, the central reverse complementary fragment comprises the 3' ITR sequence from the AAV7 genome. In some embodiments, the central reverse complementary fragment comprises the 3' ITR sequence from the AAV8 genome. In some embodiments, the central reverse complementary fragment does not comprise a functional terminal resolution site. In some embodiments, the central reverse complementary fragment is a truncated or mutated version of the 3' ITR sequence from the AAV genome. In specific embodiments, the truncated or mutated 3' ITR has the terminal resolution site (trs) disrupted. In specific embodiments, the truncated or mutated 3' ITR has the terminal resolution site (trs) removed.

[0153] ITR sequences for various AAV serotypes are known in the art. See, for example, GenBank: ITR1: NC_002077.1, nts 1-143, nts 4574-4718, ITR2: NC_001401.2, nts 1-145, nts 4535-4679, ITR3: NC_001729, nts 1-143, 4582-4726, ITR4: NC_001829.1, nts 1-146, nts 4623-4767, ITR5: NC_006152, nts 1-145, nts 4498-4642, ITR6: AF028704.1, nts 1-145, nts 4539-4683, ITR7: NC_006260.1, nts 1-145, nts 4577-4721 for the 5' (left) ITR sequences.

[0154] In specific embodiments, the single-stranded self-complementary nucleic acid molecule described herein comprises a pair of terminal self-complementary fragments located on each end, wherein at least one of the terminal self-complementary fragments comprises the 5' ITR sequence of AAV2. In some embodiments, a first terminal self-complementary fragment comprises the 5' ITR sequence of AAV2, and a second terminal self-complementary fragment comprises a sequence that is reverse complementary to the first terminal self-complementary fragment. In specific embodiments, the single-stranded self-complementary nucleic acid molecule further comprises a central self-complementary fragment comprising the 3' ITR sequence of AAV2, wherein the 3' ITR is mutated to disrupt the terminal resolution site. In specific embodiments, the single-stranded self-complementary nucleic acid molecule described herein comprises a pair of terminal self-complementary fragments located on each end, and a central self-complementary fragment located in the middle, wherein the pair of

terminal self-complementary fragments comprise the sequences of SEQ ID NO: 13 and SEQ ID NO: 36, respectively, and the central self-complementary fragment comprises the sequence of SEQ ID NO: 14.

- [0155] In specific embodiments, the single-stranded self-complementary nucleic acid molecule described herein comprises, from 5' to 3' direction, a 5' terminal self-complementary fragment as described herein, a NeuroD1 expression cassette as described herein, a central self-complementary fragment as described herein, an anti-sense region that is reverse complementary to the NeuroD1 expression cassette, and a 3' terminal self-complementary fragment as described herein. See illustration in FIG. 2A.
- [0156] In alternative embodiments, the single-stranded self-complementary nucleic acid molecule described herein comprises, from 5' to 3' direction, a 5' terminal self-complementary fragment as described herein, an anti-sense region that is reverse complementary to a NeuroD1 expression cassette, a central self-complementary fragment as described herein, a NeuroD1 expression cassette as described herein, and a 3' terminal self-complementary fragment. See illustration in FIG. 2B.
- [0157] Table 5.4 below lists the sequences of functional fragments in a single-stranded self-complementary nucleic acid molecule according to some embodiments described herein. In some embodiments, the single-stranded self-complementary nucleic acid molecule comprises, from 5' to 3' direction, a 5' terminal self-complementary fragment comprising the sequence set forth in SEQ ID NO:13, a NeuroD1 expression cassette comprising the sequence set forth in SEQ ID NO: 12, a central self-complementary fragment comprising the sequence set forth in SEQ ID NO: 14, an anti-sense region comprising the sequence set forth in SEQ ID NO: 15, and a 3' terminal self-complementary fragment comprising the sequence set forth in SEQ ID NO: 36. In specific embodiments, the 5' terminal self-complementary fragment (SEQ ID NO: 13) , the NeuroD1 expression cassette (SEQ ID NO: 12) , the central self-complementary fragment (SEQ ID NO: 14) , the anti-sense region (SEQ ID NO: 15) , and the 3' terminal self-complementary fragment (SEQ ID NO: 36) are connected directly to each other in the 5' to 3' order, and the single-stranded self-complementary nucleic acid molecule described herein comprises the sequence set forth in SEQ ID NO: 16. In some embodiments, the single-stranded self-complementary nucleic acid molecule consists of the sequence set forth in SEQ ID NO: 16.
- [0158] In alternative embodiments, the single-stranded self-complementary nucleic acid molecule comprises, from 5' to 3' direction, a 5' terminal self-complementary fragment comprising the sequence set forth in SEQ ID NO: 36, a NeuroD1 expression cassette comprising the sequence set forth in SEQ ID NO: 12, a central self-complementary fragment comprising the sequence set forth in SEQ ID NO: 14, an anti-

sense region comprising the sequence set forth in SEQ ID NO: 15, and a 3' terminal self-complementary fragment comprising the sequence set forth in SEQ ID NO: 13.

[0159] In alternative embodiments, the single-stranded self-complementary nucleic acid molecule comprises, from 5' to 3' direction, a 5' terminal self-complementary fragment comprising the sequence set forth in SEQ ID NO: 13, an anti-sense region comprising the sequence set forth in SEQ ID NO: 15, a central self-complementary fragment comprising the sequence set forth in SEQ ID NO: 14, a NeuroD1 expression cassette comprising the sequence set forth in SEQ ID NO: 12, and a 3' terminal self-complementary fragment comprising the sequence set forth in SEQ ID NO: 36.

[0160] In alternative embodiments, the single-stranded self-complementary nucleic acid molecule comprises, from 5' to 3' direction, a 5' terminal self-complementary fragment comprising the sequence set forth in SEQ ID NO: 36, an anti-sense region comprising the sequence set forth in SEQ ID NO: 15, a central self-complementary fragment comprising the sequence set forth in SEQ ID NO: 14, a NeuroD1 expression cassette comprising the sequence set forth in SEQ ID NO: 12, and a 3' terminal self-complementary fragment comprising the sequence set forth in SEQ ID NO: 13.

Table 5.4 Example of a recombinant self-complementary AAV genome encoding NeuroD1 (scAAV-NeuroD1).

scAAV-NeuroD1	
5' terminal reverse complement fragment (SEQ ID NO:13)	TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGC CGGGCGACCAAAAGGTCGCCCCGACGCCCCGGGCTTTGCCCCG GCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGG GCCAACTCCATCACTAGGGGTTCT
NeuroD1 Expression Cassette (SEQ ID NO:12)	GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACG GGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGGTTA CATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAA CGACCCCCCGCCCATTGACGTCAATAATGACGTATGTTCCC ATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGG TGGACTATTTACGGTAAACTGCCCCACTTGGCAGTACATCA AGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAAT GACGGTAAATGGCCCCGCTGGCATTATGCCCAGTACATGA CCTTATGGGACTTTCTTACTTGGCAGTACATCTACGTATTA GTCAATCGCTATTACCATGGTGATGCGGTTTGGCAGTACAT CAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTC AGTCTCCACCCCATTGACGTCAATGGGAGTTTGTGTTGGCA CCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCC GCCCCATTGACGCAAAATGGGCGGTAGGCGTGTACGGTGGG AGGTCTATATAAGCAGAGCTGTAAGTATCAAGGTTACAAG ACAGGTTTAAAGGAGACCAATAGAAAATGGGCTTGTGCGAG ACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGGT CTTACTGACATCCACTTTGCCTTCTCTCCACAGATGGTGA CCAAATCGTACAGCGAGAGTGGGCTGATGGGCGAGCCTC AGCCCCAAGGTCCTCCAAGCTGGACAGACGAGTGTCTCAG TTCTCAGGACGAGGAGCAGGAGGACAGACAAGAAGGAGGA CGACCTCGAAACCAATGAACGCAGAGGAGGACTCACTGAG GAACGGGGGAGAGGAGGAGGACGAAGATGAGGACCTGG AAGAGGAGGAAGAAAGAGGAAGAGGAGGATGACGATCAA AAGCCCCAAGAGACGCGGCCCCAAAAAGAAGAAGATGACT AAGGCTCGCCTGGAGCGTTTTAAATTGAGACGCATGAAGG CTAACGCCCCGGGAGCGGAACCGCATGCACGGACTGAACG CGGCGCTAGACAACCTGCGCAAGGTGGTGCCTTGCTATT TAAGACGCAGAAGCTGTCCAAAATCGAGACTCTGCGCTTG

	GCCAAGAAGTACATCTGGGCTCTGTCTGGAGATCCTGCGCT CAGGCAAAAGCCCAGACCTGGTCTCTCTTCGTTTCAGACGCT TTGCAAGGGCTTATCCCAACCCACCACCAACCTGGTTGCG GGCTGCCTGCAACTCAATCCTCGGACTTTTCTGCCTGAGCA GAACCAGGACATGCCCCCCCACCTGCCGACGGCCAGCGCT TCCTTCCCTGTACACCCCTACTCCTACCAGTCGCTTGGGCT GCCCAGTCCGCTTACGGTACCATGGACAGCTCCCATGTC TTCCACGTTAAGCCTCCGCGCACGCTACAGCGCAGCGC TGGAGCCCTTCTTTGAAAAGCCCTCTGACTGATTGCACCAG CCCTTCCCTTGTATGGACCCCTCAGCCCGCCGCTCAGCATCA ATGGCAACTTCTCTTTCAAACACGAACCGTCCGCCGAGTT TGAGAAAAATTATGCCTTTACCATGCACTATCCTGCAGCG ACACTGGCAGGGGGCCAAAGCCACGGATCAATCTTCTCAG GCACCGCTGCCCCCTCGCTGCGAGATCCCCATAGACAATAT TATGTCCTTCGATAGCCATTACATCATGAGCGAGTCATG AGTGCCAGCTCAATGCCATATTTTCATGATTGACTGTGCC TCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCC TTCTTGACCTGGAAGGTGCCACTCCCCTGCTCTTCTTCC AATAAAATGAGGAAATTCATTCGATTGTCTGAGTAGGTG TCAFTCTATTCCTGGGGGGTGGGGTGGGGCAGGACAGCAAG GGGGAGGATTGGGAAGAGAATAGCAGGCATGCTGGGGA
Central reverse complementary fragment (SEQ ID NO:14)	CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGG GCGACCAAAGGTGCGCCGACGCCCGGGCTTTGCCCGGGCG GCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTTGG
Anti-sense region (SEQ ID NO:15)	TCCCCAGCATGCCTGCTATTCTCTTCCCAATCCTCCCCCTT GCTGTCTGCCCCACCCACCCCCAGAAATAGAATGACAC CTACTCAGACAATGCGATGCAATTTCCTCATTTTATTAGGA AAGGACAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGG CACGGGGGAGGGGCAAACAACAGATGGCTGGCAACTAGA AGGCACAGTCAATCATGAAATATGGCATTGAGCTGGGCAC TCATGACTCGCTCATGATGTGAATGGCTATCGAAGGACAT AATATTGTCTATGGGGATCTCGCAGCGAGGGGCGAGCGGTG CCTGAGAAGATTGATCCGTGGCTTTGGGGCCCTGCCAGTG TCGCTGCAGGATAGTGCATGGTAAAGGCATAAATTTTCTC AAACCTCGGCGGACGGTTCGTGTTTGAAAGAGAAGTTGCCA TTGATGCTGAGCGGCGGGCTGAGGGGTCCATCAAAGGAA GGGCTGGTGCAATCAGTCAGAGGGCTTTCAAAGAAGGGCT CCAGCGCTGCGCTGTAGGCGTGCGGCGGAGGCTTAACGTG GAAGACATGGGAGCTGTCCATGGTACCGTAAGGCGGACT GGGCAGCCCAGGCGACTGGTAGGAGTAGGGGTGTACAGG GAAGGAAGCGCTGGCCGTCGGCAGGTGGGGGGGCATGTC CTGGTTCTGCTCAGGCAGAAAAGTCCGAGGATTGAGTTGC AGGCAGCCCGCAACCAGGTTGGTGGTGGGTGGGATAAG CCCTTGCAAAGCGTCTGAACGAAGGAGACCAGGTCTGGGC TTTTGCTGAGCGCAGGATCTCCGACAGAGCCAGATGTA GTTCTTGGCCAAGCGCAGAGTCTCGATTTTGGACAGCTTCT GCGTCTTAGAATAGCAAGGCACCACCTTGCGCAGGTTGTC TAGCGCCGCGTTCAGTCCGTGCATGCGGTTCCGCTCCCGG

	GCGTTAGCCTTCATGCGTCTCAATTTAAAAACGCTCCAGGC GAGCCTTAGTCATCTTCTTCTTTTGGGGCCGCGTCTCTTG GGCTTTTGATCGTCATCCTCCTCTTCCTCTTCTTCCTCCTCT TCCAGGTCCCTCATCTTCGTCCTCCTCCTCTCCCCCGTTCCTC AGTGAGTCCCTCCTCTGCGTTCATGGTTTCGAGGTCGTCTCTC CTTCTTGCTGCTCGCTCGTCTCCTCGTCTGAGAACTGAGAC ACTCGTCTGTCCAGCTTGGAGGACCTTGGGGCTGAGGCTC GCCCCATCAGCCCCACTCTCGCTGTACGATTTGGTCAACCATCT GTGGAGAGAAAGGCAAAGTGGATGTCAGTAAGACCAATA GGTGCCTATCAGAAACGCAAGAGTCTTCTCTGTCTCGACA AGCCCAAGTTTCTATTGGTCTCCTTAAACCTGTCTTGTAACC TTGATACTTACAGCTCTGCTTATATAGACCTCCCACCGTAC ACGCTTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTA CGACATTTTGGAAAAGTCCCCTTGATTTTGGTGCACAAAACA AACTCCCATTTGACGTCAATGGGGTGGAGACTTGGAAATCC CCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGC CAAAACCGCATCACCATTGGTAAATAGCGATGACTAAATACGT AGATGTACTGCCAAGTAGGAAAAGTCCCATAAGGTCATGTA CTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACG TCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTA CTGCCAAGTGGGCAGTTTACCGTAAATAGTCCACCCATTG ACGTCAATGGAAAAGTCCCTATTGGCGTTACTATGGGAACA TACGTCAATTATTGACGTCAATGGGCGGGGGTCTGTTGGGCG GTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCG GAACTCCATATATGGGCTATGAACTAATGACCCCGTAATT GATTACTATTAATAACTAGTCAATAATCAATGTC
3' terminal reverse complement fragment (SEQ ID NO:36)	AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCG CGCTCGCTCGCTCACTGAGGCGCGCCGGGCAAGGCCGGG CGTCGGGCGACCTTTGGTCTGCGCCGGCCTCAGTGAGCGAGC GAGCGCGCAGAGAGGGAGTGGCCAA
Full-length scAAV-NeuroD1 genomic sequence having configuration shown in FIG.2A (SEQ ID NO:16)	TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGC CGGGCGACCAAAGGTCGCCGACGCCGGGCTTTGCCCGG GCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTG GCCAACTCCATCACTAGGGGTTCTTGACATTGATTATTGA CTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCAT AGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAA ATGGCCCGCTGGCTGACCGCCCAACGACCCCGGCCATT GACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATA GGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGT AAACTGCCCCTTGGCAGTACATCAAGTGTATCATATGCC AAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCC GCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCC TACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCA TGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATA GCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCAT GACGTCAATGGGAGTTTTGTTTTGGCACCAAAATCAACGGG ACTTTCCAAAATGTCGTAACAACCTCCGCCCCATTGACGCA AATGGGCGGTAGGCGGTACGGTGGGAGGTCTATATAAGC AGAGCTGTAAGTATCAAGGTTACAAGACAGGTTTAAGGA

GACCAATAGAAACTGGGCTTGTCGAGACAGAGAAGACTC
TTGCGTTTCTGATAGGCACCTATTGGTCTTACTGACATCCA
CTTTGCCCTTCTCTCCACAGATGGTGACCAAATCGTACAGC
GAGAGTGGGCTGATGGGCGAGCCTCAGCCCCAAGGTCCCTC
CAAGCTGGACAGACGAGTGTCTCAGTTCTCAGGACGAGGA
GCACGAGGCAGACAAGAAGGAGGACGACCTCGAAACCAT
GAACGCAGAGGAGGACTCACTGAGGAACGGGGGAGAGGA
GGAGGACGAAGATGAGGACCTGGAAGAGGAGGAAGAAG
AGGAAGAGGAGGATGACGATCAAAAGCCCAAGAGACGCG
GCCCCAAAAAGAAGAAGATGACTAAGGCTCGCCTGGAGC
GTTTTAAATTGAGACGCATGAAGGCTAACGCCCGGGAGCG
GAACCGCATGCACGGACTGAACCGCGCGCTAGACAACCT
GCGCAAGGTGGTGCCTTGCTATTCTAAGACGCAGAAGCTG
TCCAAAATCGAGACTCTGCGCTTGGCCAAAGAACTACATCT
GGGCTCTGTGCGAGATCCTGCGCTCAGGCAAAAGCCCAGA
CCTGGTCTCCTTCGTTGAGACGCTTTGCAAGGGCTTATCCC
AACCACCAACCAACCTGGTTGCGGGCTGCCTGCAACTCAA
TCCTCGGACTTTTCTGCCTGAGCAGAACCAGGACATGCCC
CCCCACCTGCCGACGGCCAGCGCTTCCTTCCCTGTACACCC
CTACTCCTACCAGTCGCCCTGGGCTGCCAGTCCGCCCTTACG
GTACCATGGACAGCTCCCATGTCTTCCACGTTAAGCCTCC
GCCGCACGCCCTACAGCGCAGCGCTGGAGCCCTTCTTTGAA
AGCCCTCTGACTGATTGACACAGCCCCTTCTTTGATGGACC
CCTCAGCCCGCCGCTCAGCATCAATGGCAACTTCTCTTTCA
AACACGAACCGTCCGCCGAGTTTGAGAAAAATTATGCCTT
TACCATGCACTATCCTGCAGCGACACTGGCAGGGGGCCAA
AGCCACGGATCAATCTTCTCAGGCACCGCTGCCCTCGCT
GCGAGATCCCCATAGACAATATTATGTCTTGGATAGCCA
TTCACATCATGAGCGAGTCATGAGTGCCAGCTCAATTGCC
ATATTTCATGATTGACTGTGCCTTCTAGTTGCCAGCCATCT
GTGTGTTGCCCTCCCCCGTGCCTTCTTGACCCTGGAAGG
TGCCACTCCCACTGTCTTTTCTAATAAAATGAGGAAATT
GCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGG
GTGGGGTGGGGCAGGACAGCAAGGGGGAAGATTGGGAAG
AGAATAGCAGGCATGCTGGGGACCACTCCCTCTCTGCGCG
CTCGCTCGCTCACTGAGGCCGGCGGACCAAAGGTGCCCCG
ACGCCCCGGCTTTGCCCGGGCGGCTCAGTGAGCGAGCGA
GCGCGCAGAGAGGGAGTGGTCCCCAGCATGCCTGCTATTCT
TCTTCCCAATCCTCCCCCTTGCTGTCTGCCCCACCCACC
CCCCAGAATAGAATGACACCTACTCAGACAATGCGATGCA
ATTTCCTCATTTTATTAGGAAAGGACAGTGGGAGTGGCAC
CTTCCAGGGTCAAGGAAGGCACGGGGGAGGGGCAAAACA
CAGATGGCTGGCAACTAGAAGGCACAGTCAATCATGAAA
TATGGCATTTGAGCTGGGCACCTCACTGCTCATGATGT
GAATGGCTATCGAAGGACATAATATTGTCTATGGGGATCT
CGCAGCGAGGGGACGCGGTGCCTGAGAAGATTGATCCGT
GGCTTTGGGCCCCCTGCCAGTGTGCTGCAGGATAGTGCAT
GGTAAAGGCATAATTTTCTCAAACTCGGCGGACGGTTCTG
TGTTTGAAAGAGAAGTTGCCATTGATGCTGAGCGGCGGGC
TGAGGGGTCCATCAAAGGAAGGGCTGGTGCATCAGTCA
GAGGGCTTTCAAAGAAGGGCTCCAGCGCTGCGCTGTAGGC

	GTGCGGCGGAGGCTTAACGTGGAAGACATGGGAGCTGTC CATGGTACCGTAAAGGCGGACIGGGCAGCCCAGGCGACTG GTAGGAGTAGGGGTGTACAGGGAAGGAAGCGCTGGCCGT CGGCAGGTGGGGGGGCATGTCCTGGTTCTGCTCAGGCAGA AAAGTCCGAGGATTGAGTTGCAGGCAGCCCCGAACCAGG TTGGTGGTGGGTTGGGATAAGCCCTTGCAAAGCGTCTGAA CGAAGGAGACCAGGTCCTGGGCTTTTGCCTGAGCGCAGGAT CTCCGACAGAGCCCAGATGTAGTTCCTTGGCCAAGCGCAGA GTCTCGATTTTGGACAGCTTCTGCGTCTTAGAATAGCAAG GCACCACCTTGCGCAGGTTGTCTAGCGCCGCGTTCAGTCC GTGCATGCGGTTCCGCTCCCGGGCGTTAGCCTTCATGCGTC TCAATTIAAAACGCTCCAGGCGAGCCCTTAGTCATCTTCTTC TTTTGGGGCCGCGTCTCTTGGGCTTTTGATCGTCATCCTC CTCTTCTCTTCTTCTCTCTCTTCCAGGTCCTCATCTTCGTC CTCTTCTCTTCTCTCTCTCTCTCAGTTCCTCTCTCTGCGT TCATGGTTTCGAGGTGCTCTCTCTCTTGTCTGCCTCGTGC TCCTCGTCTTGAGAACTGAGACACTCGTCTGTCTCCAGTTG GAGGACCTTGGGGCTGAGGCTCGCCCATCAGCCCACTCTC GCTGTACGATTTGGTCACCATCTGTGGAGAGAAAGGCCAAA GTGGATGTTCAGTAAAGACCAATAGGTGCTTATCAGAAACGC AAGAGTCTTCTCTGTCTCGACAAGCCCAGTTTCTATTGGTC TCCTTAAACCTGTCTTGTAAACCTTGATACTTACAGCTCTGC TTATATAGACCTCCACCGTACACGCTACCGCCCATTTGC GTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCC GTTGATTTTGGTGCCAAAAACAAACTCCCATTTGACGTCAAT GGGGTGGAGACTTGGAATCCCCGTGAGTCAAACCGCTAT CCACGCCCATTTGATGTACTGCCAAAACCGCATCACCATGG TAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGG AAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCG GGCCATTTACCGTCATTGACGTCAATAAGGGGGCGTACTTG GCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTA CCGTAAATAGTCCACCCATTGACGTCAATGGAAAGTCCCT ATTGGCGTTACTATGGGAACATACGTCAATATTGACGTCA ATGGGCGGGGGTTCGTTGGGCGGTCAGCCAGGCGGGCCATT TACCGTAAGTTATGTAAACGCGGAACCTCATATATGGGCTA TGAACATAATGACCCCGTAATTGATTACTATTAATAACTAG TCAATAATCAATGTCAGGAACCCCTAGTGATGGAGTTGGC CACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCGCGCC GGGCAAAACCCCGGGCGTCGGGCGACCTTTGCTCGCCCGGC CTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA
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5.5 Recombinant scAAV Vectors

[0161] In some embodiments, the single-stranded self-complementary nucleic acid molecule is an artificial AAV genome that can be packaged into a AAV capsid to produce a recombinant AAV virion. In some embodiments, such recombinant AAV carries a transgene encoding NeuroD1 in its genome and is sometimes referred to as a AAV vector encoding NeuroD1 in the present disclosure.

[0162] In certain embodiments, the AAV vector encoding NeuroD1 further comprises an AAV capsid protein. In certain embodiments, the AAV capsid protein has a serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15 and AAV16, AAV. rh8, AAV. rh10, AAV. rh20, AAV. rh39, AAV. Rh74, AAV. RHM4-1, AAV. hu37, AAV. Anc80, AAV. Anc80L65, AAV. 7m8, AAV. PHP. B,

AAV2.5, AAV2tYF, AAV3B, AAV. LK03, AAV. HSC1, AAV. HSC2, AAV. HSC3, AAV. HSC4, AAV. HSC5, AAV. HSC6, AAV. HSC7, AAV. HSC8, AAV. HSC9, AAV. HSC10, AAV. HSC11, AAV. HSC12, AAV. HSC13, AAV. HSC14, AAV. HSC15, and AAV. HSC16, derivatives thereof, modifications thereof, pseudotypes thereof, and combinations thereof. In certain embodiments, the AAV capsid protein comprises or consists of an amino acid sequence that is at least about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 99.5%, or 100% homologous or identical to the amino acid sequence of viral protein 1 (VP1), viral protein 2 (VP2), or viral protein 3 (VP3) of an AAV capsid serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15 and AAV16, AAV. rh8, AAV. rh10, AAV. rh20, AAV. rh39, AAV. Rh74, AAV. RHM4-1, AAV. hu37, AAV. Anc80, AAV. Anc80L65, AAV. 7m8, AAV. PHP. B, AAV2.5, AAV2tYF, AAV3B, AAV. LK03, AAV. HSC1, AAV. HSC2, AAV. HSC3, AAV. HSC4, AAV. HSC5, AAV. HSC6, AAV. HSC7, AAV. HSC8, AAV. HSC9, AAV. HSC10, AAV. HSC11, AAV. HSC12, AAV. HSC13, AAV. HSC14, AAV. HSC15, and AAV. HSC16.

[0163] In specific embodiments, the AAV vector encoding NeuroD1 further comprises a AAV serotype 6 (AAV6) capsid. In some embodiments, the AAV6 capsid comprises at least one capsid protein comprises or consists of an amino acid sequence that is about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 99.5%, or 100% homologous or identical to the amino acid sequence of viral protein 1 (VP1), viral protein 2 (VP2), or viral protein 3 (VP3) of AAV6.

[0164] In some embodiments, the AAV6 capsid comprises a capsid protein that is a functional derivative of AAV6 VP1 having at least about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 99.5%, or 100% sequence identity to SEQ ID NO: 25. These functional derivative of AAV6 VP1 is collected referred to as "AAV6 VP1 polypeptides." In specific embodiments, the AAV6 capsid comprises a capsid protein comprising the amino acid sequence set forth in SEQ ID NO: 25.

- [0165] In some embodiments, the AAV6 capsid comprises a capsid protein that is a functional derivative of AAV6 VP2 having at least about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 99.5%, or 100% sequence identity to SEQ ID NO: 26. These functional derivative of AAV6 VP2 is collected referred to as “AAV6 VP2 polypeptides.” In specific embodiments, the AAV6 capsid comprises a capsid protein comprising the amino acid sequence set forth in SEQ ID NO: 26.
- [0166] In some embodiments, the AAV6 capsid comprises a capsid protein that is a functional derivative of AAV6 VP3 having at least about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 99.5%, or 100% sequence identity to SEQ ID NO: 27. These functional derivative of AAV6 VP3 is collected referred to as “AAV6 VP3 polypeptides.” In specific embodiments, the AAV6 capsid comprises a capsid protein comprising the amino acid sequence set forth in SEQ ID NO: 27.
- [0167] In some embodiments, the AAV6 capsid comprises an AAV VP1 polypeptide and an AAV VP2 polypeptide. In some embodiments, the AAV6 capsid comprises an AAV VP1 polypeptide and an AAV VP3 polypeptide. In some embodiments, the AAV6 capsid comprises an AAV VP2 polypeptide and an AAV VP3 polypeptide. In some embodiments, the AAV6 capsid comprises an AAV VP1 polypeptide, an AAV VP2 polypeptide, and an AAV VP3 polypeptide.
- [0168] In yet a particular embodiment, the AAV vector encoding NeuroD1 further comprises a AAV6 capsid, wherein the AAV6 capsid comprises a VP1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 25, a VP2 polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 26, and a VP3 polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 27.
- [0169] In certain embodiments, the AAV vector comprises capsid of Anc80 or Anc80L65, as described in Zinn et al., 2015, Cell Rep. 12 (6) : 1056-1068, the content of which is incorporated by reference in its entirety. In certain embodiments, the AAV vector comprises the capsid with one of the following amino acid insertions: LGETTRP or LALGETTRP, as described in U.S. Patent Nos. 9,193,956; 9,458,517; and 9,587,282 and U.S. patent application publication no. 2016/0376323, the content of each of which is incorporated herein by reference in its entirety. In certain embodiments, the AAV vector comprises the capsid of AAV. 7m8, as described in U.S. Patent Nos. 9,193,956; 9,458,517; and 9,587,282 and U.S. patent application pub-

lication no. 2016/0376323, the content of each of which is incorporated herein by reference in its entirety. In certain embodiments, the AAV vector comprises any AAV capsid disclosed in U.S. Patent No. 9,585,971 (e.g., AAV-PHP. B), the content of which is incorporated by reference in its entirety. In certain embodiments, the AAV vector comprises any AAV capsid disclosed in U.S. Patent No. 9,840,719 and WO 2015/013313, such as AAV. Rh74 and RHM4-1, the content of each of which is incorporated herein by reference in its entirety. In certain embodiments, the AAV vector comprises any AAV capsid disclosed in International Publication No. WO 2014/172669, such as AAV rh. 74, the content of which is incorporated herein by reference in its entirety. In certain embodiments, the AAV vector comprises the capsid of AAV2/5, as described in Georgiadis et al., 2016, Gene Therapy 23: 857-862 and Georgiadis et al., 2018, Gene Therapy 25: 450, the content of each of which is incorporated by reference in its entirety. In certain embodiments, the AAV vector comprises any AAV capsid disclosed in International Publication No. WO 2017/070491, such as AAV2tYF, the content of which is incorporated herein by reference in its entirety. In certain embodiments, the AAV vector comprises the capsids of AAVLK03 or AAV3B, as described in Puzzo et al., 2017, Sci. Transl. Med. 29 (9) : 418, the content of which is incorporated by reference in its entirety. In certain embodiments, the AAV vector comprises any AAV capsid disclosed in U.S. Patent Nos. 8,628,966; 8,927,514; and 9,923,120 and International Publication No. WO 2016/049230, such as HSC1, HSC2, HSC3, HSC4, HSC5, HSC6, HSC7, HSC8, HSC9, HSC10, HSC11, HSC12, HSC13, HSC14, HSC15, or HSC16, the content of each of which is incorporated by reference in its entirety.

[0170] In certain embodiments, the AAV vector comprises an AAV capsid disclosed in any of the following patents and patent applications, the content of each of which is incorporated herein by reference in its entirety: U.S. Patent Nos. 7,282,199; 7,906,111; 8,524,446; 8,999,678; 8,628,966; 8,927,514; 8,734,809; 9,284,357; 9,409,953; 9,169,299; 9,193,956; 9,458,517; and 9,587,282; U.S. Patent Publication Nos. 2015/0374803; 2015/0126588; 2017/0067908; 2013/0224836; 2016/0215024; 2017/0051257; and International Patent Publication Nos. WO2015191508; WO2015121501. In certain embodiments, the AAV vector comprises a capsid protein that is at least about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 99.5%, or 100% homologous or identical to the amino acid sequence of the VP1, VP2, or VP3 of an AAV capsid disclosed in any of the following patents and patent applications, the content each of which is incorporated herein by reference in its

entirety: U.S. Patent Nos. 7,282,199; 7,906,111; 8,524,446; 8,999,678; 8,628,966; 8,927,514; 8,734,809; 9,284,357; 9,409,953; 9,169,299; 9,193,956; 9,458,517; and 9,587,282; U.S. Patent Publication Nos. 2015/0374803; 2015/0126588; 2017/0067908; 2013/0224836; 2016/0215024; 2017/0051257; and International Patent Publication Nos. WO2015191508; WO2015121501.

[0171] In certain embodiments, the AAV vector comprises a capsid protein disclosed in International Patent Publication Nos. WO 2003/052051 (see, e.g., SEQ ID NO: 2 of WO 2003/052051), WO 2005/033321 (see, e.g., SEQ ID NOs: 123 and 88 of WO 2005/033321), WO 03/042397 (see, e.g., SEQ ID NOs: 2, 81, 85, and 97 of WO 03/042397), WO 2006/068888 (see, e.g., SEQ ID NOs: 1 and 3-6 of WO 2006/068888), WO 2006/110689 (see, e.g., SEQ ID NOs: 5-38 of WO 2006/110689), WO2009/104964 (see, e.g., SEQ ID NOs: 1-5, 7, 9, 20, 22, 24 and 31 of WO2009/104964), WO 2010/127097 (see, e.g., SEQ ID NOs: 5-38 of WO 2010/127097), and WO 2015/191508 (see, e.g., SEQ ID NOs: 80-294 of WO 2015/191508), and U.S. Patent Publication No. 20150023924 (see, e.g., SEQ ID NOs: 1, 5-10 of 20150023924), the contents of each of which is herein incorporated by reference in its entirety. In certain embodiments, the AAV vector comprises a capsid protein at least about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 99.5%, or 100% homologous or identical to the amino acid sequence of the VP1, VP2, or VP3 protein of an AAV capsid disclosed in International Patent Publication Nos. WO 2003/052051 (see, e.g., SEQ ID NO: 2 of WO 2003/052051), WO 2005/033321 (see, e.g., SEQ ID NOs: 123 and 88 of WO 2005/033321), WO 03/042397 (see, e.g., SEQ ID NOs: 2, 81, 85, and 97 of WO 03/042397), WO 2006/068888 (see, e.g., SEQ ID NOs: 1 and 3-6 of WO 2006/068888), WO 2006/110689 (see, e.g., SEQ ID NOs: 5-38 of WO 2006/110689), WO2009/104964 (see, e.g., SEQ ID NOs: 1-5, 7, 9, 20, 22, 24 and 31 of WO2009/104964), WO 2010/127097 (see, e.g., SEQ ID NOs: 5-38 of WO 2010/127097), and WO 2015/191508 (see, e.g., SEQ ID NOs: 80-294 of WO 2015/191508), and U.S. Patent Publication No. 20150023924 (see, e.g., SEQ ID NOs: 1, 5-10 of 20150023924).

[0172] In certain embodiments, the AAV vector comprises a pseudotyped AAV capsid. In certain embodiments, the pseudotyped AAV capsids are rAAV2/8 or rAAV2/9 pseudotyped AAV capsids. Methods for producing and using pseudotyped AAV vectors are known in the art (see, e.g., Duan et al., J. Virol., 75: 7662-7671 (2001); Halbert et al., J. Virol., 74: 1524-1532 (2000); Zolotukhin et al., Methods 28: 158-167

(2002) ; and Auricchio et al., Hum. Molec. Genet. 10: 3075-3081, (2001) , the content of each of which is incorporated by reference in its entirety) .

[0173] In certain embodiments, the AAV vector comprises a capsid comprising a capsid protein chimeric of two or more AAV capsid serotypes. In certain embodiments, the capsid protein is a chimeric of two or more AAV capsid proteins of AAV serotypes selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15 and AAV16, AAV. rh8, AAV. rh10, AAV. rh20, AAV. rh39, AAV. Rh74, AAV. RHM4-1, AAV. hu37, AAV. Anc80, AAV. Anc80L65, AAV. 7m8, AAV. PHP. B, AAV2.5, AAV2tYF, AAV3B, AAV. LK03, AAV. HSC1, AAV. HSC2, AAV. HSC3, AAV. HSC4, AAV. HSC5, AAV. HSC6, AAV. HSC7, AAV. HSC8, AAV. HSC9, AAV. HSC10, AAV. HSC11, AAV. HSC12, AAV. HSC13, AAV. HSC14, AAV. HSC15, and AAV. HSC16.

[0174] In certain embodiments, the AAV vector comprises an AAV capsid protein chimeric of AAV8 capsid protein and one or more AAV capsid proteins from an AAV serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15 and AAV16, AAV. rh8, AAV. rh10, AAV. rh20, AAV. rh39, AAV. Rh74, AAV. RHM4-1, AAV. hu37, AAV. Anc80, AAV. Anc80L65, AAV. 7m8, AAV. PHP. B, AAV2.5, AAV2tYF, AAV3B, AAV. LK03, AAV. HSC1, AAV. HSC2, AAV. HSC3, AAV. HSC4, AAV. HSC5, AAV. HSC6, AAV. HSC7, AAV. HSC8, AAV. HSC9, AAV. HSC10, AAV. HSC11, AAV. HSC12, AAV. HSC13, AAV. HSC14, AAV. HSC15, and AAV. HSC16. In certain embodiments, the AAV vector comprises an AAV capsid protein chimeric of AAV8 capsid protein and one or more AAV capsid proteins from an AAV serotype selected from the group consisting of AAV1, AAV2, AAV5, AAV6, AAV7, AAV9, AAV10, AAVrh. 8, and AAVrh. 10.

[0175] In certain embodiments, the AAV vector comprises an AAV capsid protein chimeric of AAV9 capsid protein the capsid protein of one or more AAV capsid serotypes selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15 and AAV16, AAV. rh8, AAV. rh10, AAV. rh20, AAV. rh39, AAV. Rh74, AAV. RHM4-1, AAV. hu37, AAV. Anc80, AAV. Anc80L65, AAV. 7m8, AAV. PHP. B, AAV2.5, AAV2tYF, AAV3B, AAV. LK03, AAV. HSC1, AAV. HSC2, AAV. HSC3, AAV. HSC4, AAV. HSC5, AAV. HSC6, AAV. HSC7, AAV. HSC8, AAV. HSC9, AAV. HSC10, AAV. HSC11, AAV. HSC12, AAV. HSC13, AAV. HSC14, AAV. HSC15, and AAV. HSC16

[0176] In certain embodiments, the AAV vectors comprises a mosaic capsid. In certain embodiments, the mosaic capsid comprises a mixture of viral capsid proteins from

different AAV serotypes. In certain embodiments, the mosaic capsid comprises capsid proteins of serotypes selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15 and AAV16, AAV. rh8, AAV. rh10, AAV. rh20, AAV. rh39, AAV. Rh74, AAV. RHM4-1, AAV. hu37, AAV. Anc80, AAV. Anc80L65, AAV. 7m8, AAV. PHP. B, AAV2.5, AAV2tYF, AAV3B, AAV. LK03, AAV. HSC1, AAV. HSC2, AAV. HSC3, AAV. HSC4, AAV. HSC5, AAV. HSC6, AAV. HSC7, AAV. HSC8, AAV. HSC9, AAV. HSC10, AAV. HSC11, AAV. HSC12, AAV. HSC13, AAV. HSC14, AAV. HSC15, and AAV. HSC16. In certain embodiments, the mosaic capsid comprises capsid proteins of serotypes selected from the group consisting of AAV1, AAV2, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAVrh. 8, and AAVrh. 10.

[0177] In certain embodiments, the AAV vector comprises a pseudotyped AAV vector. In certain embodiments, the pseudotyped AAV vector comprises a capsid protein of an AAV serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15 and AAV16, AAV. rh8, AAV. rh10, AAV. rh20, AAV. rh39, AAV. Rh74, AAV. RHM4-1, AAV. hu37, AAV. Anc80, AAV. Anc80L65, AAV. 7m8, AAV. PHP. B, AAV2.5, AAV2tYF, AAV3B, AAV. LK03, AAV. HSC1, AAV. HSC2, AAV. HSC3, AAV. HSC4, AAV. HSC5, AAV. HSC6, AAV. HSC7, AAV. HSC8, AAV. HSC9, AAV. HSC10, AAV. HSC11, AAV. HSC12, AAV. HSC13, AAV. HSC14, AAV. HSC15, and AAV. HSC16. In certain embodiments, the pseudotyped AAV vector are AAV2/8 or AAV2/9 pseudotyped vectors. Methods for producing and using pseudotyped AAV vectors are known in the art (see, e.g., Duan et al., *J. Virol.*, 75: 7662-7671 (2001) ; Halbert et al., *J. Virol.*, 74: 1524-1532 (2000) ; Zolotukhin et al., *Methods* 28: 158-167 (2002) ; and Auricchio et al., *Hum. Molec. Genet.* 10: 3075-3081, (2001, the content of each of which is incorporated herein by reference in its entirety) .

[0178] Nucleotide sequences of AAV vectors and methods of making thereof are taught, for example, in U.S. Patent Nos. 7,282,199; 7,906,111; 8,524,446; 8,999,678; 8,628,966; 8,927,514; 8,734,809; US 9,284,357; 9,409,953; 9,169,299; 9,193,956; 9,458,517; and 9,587,282; U.S. Patent Publication Nos. 2015/0374803; 2015/0126588; 2017/0067908; 2013/0224836; and 2016/0215024; 2017/0051257; 2015/0023924; International Patent Publication Nos. WO2015191508; WO2015121501; WO 2003/052051, WO 2005/033321, WO 03/042397, WO 2006/068888, WO 2006/110689, WO2009/104964, WO 2010/127097, and WO 2015/191508, the content of each of which is incorporated herein by reference in its entirety.

5.6 Methods and Compositions for Making Recombinant AAV

- [0179] In one aspect, the present disclosure provides plasmids comprising a presently disclosed expression cassettes (e.g., expression cassettes disclosed in Section Error! Reference source not found. (NeuroD1 Expression Cassette) of the present disclosure) . The presently disclosed plasmids can be used for producing AAV vectors (e.g., AAV vectors disclosed in Section 5.5 (Recombinant scAAV Vectors) of the present disclosure) by being delivered into host cell for AAV packaging.
- [0180] In some embodiments, provided herein is a gene-of-interest (GOI) plasmid that carries a transgene encoding a NeuroD1 polypeptide. In some embodiments, the GOI plasmid comprises a NeuroD1 expression cassette as described in Section 5.3 (NeuroD1 Expression Cassette) and a pair of AAV ITR sequences flanking the NeuroD1 expression cassette.
- [0181] In some embodiments, the pair of AAV ITRs are independently selected from the genomic ITR sequences of AAV serotypes AAV1, AAV2, AAV2tYF, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAVrh10, AAV. rh20, AAV. rh39, AAV. Rh74, AAV. RHM4-1, AAV. hu37, AAV. Anc80, AAV. An-c80L65, rAAV. 7m8, AAV. PHP. B, AAV. PHP. eB, AAV2.5, AAV2tYF, AAV3B, AAV. LK03, AAV. HSC1, AAV. HSC2, AAV. HSC3, AAV. HSC4, AAV. HSC5, AAV. HSC6, AAV. HSC7, AAV. HSC8, AAV. HSC9, AAV. HSC10 , AAV. HSC11, AAV. HSC12, AAV. HSC13, AAV. HSC14, AAV. HSC15, and AAV. HSC16.
- [0182] In some embodiments, the ITR sequence located 5' to the NeuroD1 expression cassette comprises a functional terminal resolution site. In some embodiments, the ITR sequence located 5' to the NeuroD1 expression cassette comprises the full-length 5' ITR sequence of the AAV genome. In some embodiments, the ITR sequence located 5' to the NeuroD1 expression cassette comprises the wild-type 5' ITR sequence of the AAV genome. In some embodiments, the ITR sequence located 3' to the NeuroD1 expression cassette does not comprise a functional trs. In some embodiments, the ITR sequence located 3' to the NeuroD1 expression cassette is a truncated version of the 3' ITR sequence of the AAV genome that removes the trs. In some embodiments, the ITR sequence located 3' to the NeuroD1 expression cassette is a mutated version of the 3' ITR sequence of the AAV genome that disrupts the trs function. See illustration in FIG. 2A.
- [0183] In alternative embodiments, the ITR sequence located 3' to the NeuroD1 expression cassette comprises a functional terminal resolution site. In some embodiments, the ITR sequence located 3' to the NeuroD1 expression cassette comprises the full-length 3' ITR sequence of the AAV genome. In some embodiments, the ITR sequence located 3' to the NeuroD1 expression cassette comprises the wild-type 3' ITR sequence of the AAV genome. In some embodiments, the ITR sequence located 5' to the NeuroD1 expression cassette does not comprise a functional trs. In some embodiments, the ITR

sequence located 5' to the NeuroD1 expression cassette is a truncated version of the 5' ITR sequence of the AAV genome that removes the trs. In some embodiments, the ITR sequence located 5' to the NeuroD1 expression cassette is a mutated version of the 5' ITR sequence of the AAV genome that disrupts the trs function. See illustration in FIG. 2B.

[0184] In some embodiments, the ITR sequence located 5' to the NeuroD1 expression cassette (i.e., the 5' ITR sequence in the plasmid) is selected from the genomic ITR sequences of AAV serotypes 1 to 8. The 5' ITR sequence in the plasmid comprises the 5' ITR sequence from the AAV1 genome. The 5' ITR sequence in the plasmid comprises the 5' ITR sequence from the AAV2 genome. The 5' ITR sequence in the plasmid comprises the 5' ITR sequence from the AAV3 genome. The 5' ITR sequence in the plasmid comprises the 5' ITR sequence from the AAV4 genome. The 5' ITR sequence in the plasmid comprises the 5' ITR sequence from the AAV5 genome. The 5' ITR sequence in the plasmid comprises the 5' ITR sequence from the AAV6 genome. The 5' ITR sequence in the plasmid comprises the 5' ITR sequence from the AAV7 genome. The 5' ITR sequence in the plasmid comprises the 5' ITR sequence from the AAV8 genome. In some embodiments, the 5' ITR sequence in the plasmid comprises a functional terminal resolution site. In some embodiments, the 5' ITR sequence in the plasmid comprises the full-length 5' ITR sequence of the AAV genome. In some embodiments, the 5' ITR sequence in the plasmid comprises the wild-type 5' ITR sequence of the AAV genome.

[0185] In some embodiments, the ITR sequence located 3' to the NeuroD1 expression cassette (i.e., the 3' ITR sequence in the plasmid) is selected from the genomic ITR sequences of AAV serotypes 1 to 8. In some embodiments, the 3' ITR sequence in the plasmid comprises the 3' ITR sequence from the AAV1 genome. In some embodiments, the 3' ITR sequence in the plasmid comprises the 3' ITR sequence from the AAV2 genome. In some embodiments, the 3' ITR sequence in the plasmid comprises the 3' ITR sequence from the AAV3 genome. In some embodiments, the 3' ITR sequence in the plasmid comprises the 3' ITR sequence from the AAV4 genome. In some embodiments, the 3' ITR sequence in the plasmid comprises the 3' ITR sequence from the AAV5 genome. In some embodiments, the 3' ITR sequence in the plasmid comprises the 3' ITR sequence from the AAV6 genome. In some embodiments, the 3' ITR sequence in the plasmid comprises the 3' ITR sequence from the AAV7 genome. In some embodiments, the 3' ITR sequence in the plasmid comprises the 3' ITR sequence from the AAV8 genome. In some embodiments, the 3' ITR sequence in the plasmid does not comprise a functional terminal resolution site. In some embodiments, the 3' ITR sequence in the plasmid is a truncated version of the 3' ITR sequence of the AAV genome that removes the trs. In some embodiments, the 3'

ITR sequence in the plasmid is a mutated version of the 3' ITR sequence of the AAV genome that disrupts the trs function. ITR sequences for various AAV serotypes are known in the art. See, for example, GenBank: ITR1: NC_002077.1, nts 1-143, ITR2: NC_001401.2, nts 1-145, ITR3: JB292182.1, nts 1-143, ITR4: NC_001829.1, nts 1-146, ITR6: AF028704.1, nts 1-145, ITR7: NC_006260.1, nts 1-145, for the 5' (left) ITR sequences.

- [0186] In specific embodiments, the plasmid comprises a pair of ITR sequences located on each end of an NeuroD1 expression cassette, and wherein the ITR located 5' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 13, and the ITR located 3' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 14, and wherein the NeuroD1 expression cassette comprises a coding sequence that encodes a NeuroD1 polypeptide comprising an amino acid sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO: 1.
- [0187] In yet a specific embodiment, the plasmid comprises a pair of ITR sequences located on each end of an NeuroD1 expression cassette, and wherein the ITR located 5' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 13, and the ITR located 3' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 14, and wherein the NeuroD1 expression cassette comprises a coding sequence comprising the sequence set forth in SEQ ID NO: 5 or codon-optimized variant thereof. In yet a specific embodiment, the NeuroD1 expression cassette further comprises one or more regulatory elements operably linked to the coding sequence. In specific embodiments, the regulatory elements are one or more selected from a CMV enhancer sequence, a CMV promoter sequence, a chimeric intron, and a polyadenylation signal.
- [0188] In yet a specific embodiment, the plasmid comprises a pair of ITR sequences located on each end of an NeuroD1 expression cassette, and wherein the ITR located 5' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 13, and the ITR located 3' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 14, and wherein the NeuroD1 expression cassette comprises a coding sequence comprising the sequence set forth in SEQ ID NO: 5 or codon-optimized variant thereof. In yet a specific embodiment, the NeuroD1 expression cassette further comprises one or more regulatory elements operably linked to the coding sequence. In specific embodiments, the regulatory elements are one or more selected from a CMV enhancer sequence, a CMV promoter sequence, a chimeric intron, and a polyadenylation signal. In specific embodiments, the regulatory elements are one or more selected from a CMV enhancer sequence comprising the sequence set forth in SEQ ID NO: 6, a CMV promoter sequence comprising the sequence set forth in SEQ

ID NO: 7, a chimeric intron comprising the sequence set forth in SEQ ID NO: 8, and a polyadenylation signal comprising the sequence set forth in SEQ ID NO: 9.

[0189] In yet a specific embodiment, the plasmid comprises a pair of ITR sequences located on each end of an NeuroD1 expression cassette, and wherein the ITR located 5' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 13, and the ITR located 3' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 14, and wherein the NeuroD1 expression cassette comprises the sequence set forth in SEQ ID NO: 12. In yet a specific embodiment, the plasmid comprises a pair of ITR sequences located on each end of an NeuroD1 expression cassette, and wherein the ITR located 5' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 13, and the ITR located 3' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 14, and wherein the NeuroD1 expression cassette consists of the sequence set forth in SEQ ID NO: 12.

[0190] In alternative embodiments, the plasmid comprises a pair of ITR sequences located on each end of an NeuroD1 expression cassette, and wherein the ITR located 3' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 13, and the ITR located 5' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 14, and wherein the NeuroD1 expression cassette comprises a coding sequence that encodes a NeuroD1 polypeptide comprising an amino acid sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO: 1.

[0191] In yet a specific alternative embodiment, the plasmid comprises a pair of ITR sequences located on each end of an NeuroD1 expression cassette, and wherein the ITR located 3' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 13, and the ITR located 5' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 14, and wherein the NeuroD1 expression cassette comprises a coding sequence comprising the sequence set forth in SEQ ID NO: 5 or codon-optimized variant thereof. In yet a specific embodiment, the NeuroD1 expression cassette further comprises one or more regulatory elements operably linked to the coding sequence. In specific embodiments, the regulatory elements are one or more selected from a CMV enhancer sequence, a CMV promoter sequence, a chimeric intron, and a polyadenylation signal.

[0192] In yet a specific alternative embodiment, the plasmid comprises a pair of ITR sequences located on each end of an NeuroD1 expression cassette, and wherein the ITR located 3' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 13, and the ITR located 5' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 14, and wherein the NeuroD1 expression cassette comprises a coding sequence comprising the sequence set forth in SEQ ID NO: 5 or codon-optimized variant thereof. In yet a specific alternative embodiment, the NeuroD1 ex-

pression cassette further comprises one or more regulatory elements operably linked to the coding sequence. In specific embodiments, the regulatory elements are one or more selected from a CMV enhancer sequence, a CMV promoter sequence, a chimeric intron, and a polyadenylation signal. In specific embodiments, the regulatory elements are one or more selected from a CMV enhancer sequence comprising the sequence set forth in SEQ ID NO: 6, a CMV promoter sequence comprising the sequence set forth in SEQ ID NO: 7, a chimeric intron comprising the sequence set forth in SEQ ID NO: 8, and a polyadenylation signal comprising the sequence set forth in SEQ ID NO: 9.

[0193] In yet a specific alternative embodiment, the plasmid comprises a pair of ITR sequences located on each end of an NeuroD1 expression cassette, and wherein the ITR located 3' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 13, and the ITR located 5' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 14, and wherein the NeuroD1 expression cassette comprises the sequence set forth in SEQ ID NO: 12. In yet a specific alternative embodiment, the plasmid comprises a pair of ITR sequences located on each end of an NeuroD1 expression cassette, and wherein the ITR located 3' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 13, and the ITR located 5' to the NeuroD1 expression cassette comprises the sequence of SEQ ID NO: 14, and wherein the NeuroD1 expression cassette consists of the sequence set forth in SEQ ID NO: 12.

[0194] In some embodiments, the plasmid further comprises a backbone sequence. Any suitable plasmid backbone known in the art for the production of AAV vectors can be used with the presently disclosed subject matter, and an exemplary plasmid backbone sequence is provided in Table 6.1.1 (A) (see e.g., SEQ ID NO: 17) .

[0195] The present disclosure provides host cells comprising a presently disclosed plasmid. Any suitable host cells for AAV vector production can be used with the presently disclosed subject matter. In certain embodiments, the host cell is a mammalian cell. In certain embodiments, the host cell from humans, monkeys, mice, rats, rabbits, or hamsters. In certain embodiments, the host cell is an insect cell.

[0196] Non-limiting examples of suitable host cells that can be used with the presently disclosed subject matter include A549 cells, WEHI cells, 10T1/2 cells, MDCK cells, COS1 cells, COS7 cells, BSC 1 cells, BSC 40 cells, BMT 10 cells, VERO cells, W138 cells, Saos cells, C2C12 cells, L cells, HT1080 cells, HepG2 cells, HeLa cells, HEK293 cells, HEK293 derived cells (e.g., HEK293T cells, HEK293F cells) , CHO cells, CHO-K1 cells, CHO derived cells, EB66 cells, BSC cells, HepG2 cells, LLC-MK cells, CV-1 cells, COS cells, MDBK cells, MDCK cells, CRFK cells, RAF cells, RK cells, TCMK-1 cells, LLCPK cells, PK15 cells, LLC-RK cells, BHK cells, BHK-21 cells, NS-1 cells, MRC-5 cells, WI-38 cells, 3T3 cells, 293 cells, RK cells, Per. C6 cells, chicken embryo cells, SF-9 cells, primary fibroblasts, primary he-

patocytes, primary myoblast cells, primary hippocampal neurons, primary cerebral cortical neurons, primary cerebellar neurons, and primary striatal neurons.

[0197] Any suitable methods known in the art for producing recombinant AAV can be used with the presently disclosed subject matter for producing the recombinant AAV as described herein (e.g., recombinant AAV disclosed in Section 5.5 (Recombinant scAAV Vectors) of the present disclosure). In certain embodiments, the methods comprise: (a) transfecting a host cell described herein with a presently disclosed GOI plasmid, (b) culturing the host cell in a culturing medium; and (c) isolating the recombinant AAV virions from the culturing medium. In certain embodiments, the methods further comprise transfecting the host cell with a plasmid comprising an expression cassette encoding AAV rep proteins and capsid proteins. In certain embodiments, the methods further comprise transfecting the host cell with a plasmid encoding adenovirus regions (e.g., VA, E2A and E4) that mediate AAV vector replication. In certain embodiments, the methods comprise: (a) culturing a host cell comprising a cis expression cassette (e.g., expression cassettes disclosed in Section 5.3 (NeuroD1 Expression Cassette) of the present disclosure) in a culture medium, and (b) isolating the recombinant AAV virions from the cell culture. In certain embodiments, the host cell further comprises (ii) a trans expression cassette encoding one or more AAV rep proteins and capsid proteins. In certain embodiments, the host cell further comprises (iii) nucleic acid sequence encoding adenovirus regions (e.g., VA, E2A and E4) that mediate AAV vector replication. Exemplary plasmid sequences for the GOI plasmid, rep-cap packaging plasmid and helper plasmid that can be used in connection with the present disclosure are provided in Table 6.1.2 (B).

[0198] Exemplary methods of producing recombinant AAV vectors are disclosed in International Patent Publication Nos. WO20220033842 and WO2019079496, the content of each of which is incorporated by reference in its entirety.

[0199] Genome copy titers of the recombinant AAV vectors may be determined, for example, by TAQMAN® analysis. Virions may be recovered, for example, by CsCl₂ sedimentation. Alternatively, baculovirus expression systems in insect cells may be used to produce AAV vectors. For a review, see Aponte-Ubillus et al., 2018, Appl. Microbiol. Biotechnol. 102: 1045-1054, which is incorporated by reference herein in its entirety for manufacturing techniques.

[0200] In vitro assays, e.g., cell culture assays, can be used to measure coding nucleotide sequence expression from the recombinant AAV vector, thus indicating, e.g., potency of the recombinant vector. For example, the HeLa cell, a cell line derived from human cervical cancer cells (available from ATCC®), can be used to assess coding nucleotide sequence expression. Alternatively, cell lines derived from liver or muscle or other cell types may be used, for example, but not limited, to HuH-7, HEK293, fibrosarcoma

HT-1080, HKB-11, C2C12 myoblasts, and CAP cells. Once expressed, characteristics of the expressed product (e.g., protein) can also be determined, including serum half-life, functional activity of the protein (e.g., enzymatic activity or binding to a target), determination of the glycosylation and tyrosine sulfation patterns, and other assays known in the art for determining protein characteristics.

5.7 Pharmaceutical Composition and Kit

[0201] In one aspect, the present disclosure provides a pharmaceutical composition comprising a recombinant AAV vector (e.g., recombinant AAV vectors disclosed in Section 5.5 (Recombinant scAAV Vectors)) and a pharmaceutically acceptable carrier.

[0202] In certain embodiments, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. As used herein, the term “carrier” refers to a diluent, an adjuvant (e.g., Freund’s complete and incomplete adjuvant), an excipient, or vehicle with which the AAV vector is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, including, e.g., peanut oil, soybean oil, mineral oil, sesame oil or the like. Water is a common carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include but not limited to starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Additional examples of pharmaceutically acceptable carriers, excipients, and stabilizers include, but are not limited to, buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin and gelatin; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEENTM, polyethylene glycol (PEG), and PLURONICSTM as known in the art. In certain embodiments, the pharmaceutical composition further comprises a lubricant, a wetting agent, a sweetener, a flavoring agent, an emulsifier, a suspending agent, or a preservative, in addition to the above ingredients. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations or the like.

- [0203] In certain embodiments, the pharmaceutical composition is provided for use in accordance with the presently disclosed methods of treatment (e.g., methods of treatment disclosed in Section Error! Reference source not found. (Method of Treatment) of the present disclosure), said pharmaceutical compositions comprise a therapeutically and/or prophylactically effective amount of the presently disclosed recombinant AAV vector and a pharmaceutically acceptable carrier.
- [0204] In certain embodiments, the AAV vector is substantially purified (i.e., substantially free from substances that limit its effect or produce undesired side-effects). In certain embodiments, the subject receiving the pharmaceutical composition is a mammal such as non-primate (e.g., cows, pigs, horses, cats, dogs, rats etc.) and a primate (e.g., monkey such as, a cynomolgus monkey and a human). In certain embodiments, the subject is a human.
- [0205] In some embodiments, the pharmaceutical composition is in a fluidic formulation. In some embodiments, the pharmaceutical composition is a solution. In some embodiments, the pharmaceutical composition comprises a recombinant AAV described in Section 5.5 (Recombinant scAAV Vectors), and further comprises:
- (a) potassium chloride,
 - (b) potassium phosphate monobasic,
 - (c) sodium chloride,
 - (d) sodium phosphate dibasic anhydrous, and
 - (e) poloxamer 188, polysorbate 20, or polysorbate 80.
- [0206] In some embodiments, the pharmaceutical composition comprises a recombinant AAV described in Section 5.5 (Recombinant scAAV Vectors), and further comprises:
- (a) sodium chloride at a concentration of about 180 mM;
 - (b) sodium phosphate at a concentration of about 10 mM; and
 - (c) poloxamer 188 at a concentration of about 0.001% weight/volume (0.01 g/L); and
- wherein the pH of the pharmaceutical composition is about 7.3.
- [0207] In some embodiments, the pharmaceutical composition comprises a recombinant AAV described in Section 5.5 (Recombinant scAAV Vectors), and further comprises:
- (a) sodium chloride at a concentration of about 200 mM;
 - (b) magnesium chloride at a concentration of about 1 mM;
 - (c) Tris hydrochloride at a concentration of about 20 mM, and
 - (d) poloxamer 188 at a concentration of about 0.005% weight/volume (0.05 g/L); and
- wherein the pH of the pharmaceutical composition is about 8.0.
- [0208] In some embodiments, the pharmaceutical composition comprises a recombinant AAV described in Section 5.5 (Recombinant scAAV Vectors), and further comprises:
- (a) sodium chloride at a concentration of about 150 mM;
 - (b) calcium chloride at a concentration of about 1.4 mM;

(c) magnesium chloride at a concentration of about 0.8 mM,
(d) sodium phosphate at a concentration of about 1 mM, and
(e) poloxamer 188 at a concentration of about 0.001% weight/volume (0.01 g/L) ; and
wherein the pH of the pharmaceutical composition is about 7.4.

[0209] The present disclosure further provides kits that for selectively expressing a coding nucleic acid in glioma (e.g., glioblastoma) cells of a subject. The present disclosure also provides kits for treating glioma (e.g., glioblastoma) in a subject in need thereof.

[0210] In certain embodiment, the kit comprises the presently disclosed recombinant AAV vector, e.g., in a container. Such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments. Optionally associated with such container (s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use, or sale for human administration.

[0211] In certain embodiments, the kit further comprises instructions for administering to a subject having glioma. The instructions generally include information about the use of the composition for the treatment and/or prevention of glioma. In certain embodiments, the instructions include at least one of the following: description of the therapeutic agent; dosage schedule and administration for treatment or prevention of glioma or symptoms thereof; precautions; warnings; indications; counter-indications; over-dosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present) , or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

5.8 Method of Treatment

[0212] The present disclosure provides methods for selectively expressing an encoding nucleic acid in glial cells, including glial cells that are undergoing various stages of pathogenic neoplasm, including glioma cells. The present disclosure also provides methods for treating glioma in a subject in need thereof. In certain embodiments, the methods comprise delivering to the subject a presently disclosed recombinant AAV (e.g., AAV vectors disclosed in Section 5.5 (Recombinant scAAV Vectors) of the present disclosure) . In certain embodiments, the methods comprise delivering to the subject a presently disclosed pharmaceutical composition comprising recombinant AAV (e.g., pharmaceutical composition disclosed in Section 5.7 (Formulation of Pharmaceutical Composition) of the present disclosure) .

[0213] In some embodiments, the methods of treating a glioma comprise delaying, preventing, treating, and/or managing the disease or disorder. In certain embodiments,

the methods prevent occurrence or recurrence of the disease or disorder. In certain embodiments, the methods alleviate one or more symptoms of the disease or disorder. In certain embodiments, the methods diminish any direct or indirect pathological consequences of the disease or disorder. In certain embodiments, the methods decrease the rate of disease progression. In certain embodiments, the methods delay remission or improves prognosis of the disease or disorder.

- [0214] In some embodiments, the method for treating glioma comprises administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising a recombinant AAV, wherein the recombinant AAV comprises a self-complementary genome comprising a transgene encoding a NeuroD1 polypeptide.
- [0215] In some embodiments, the self-complementary genome is a single-stranded DNA molecule comprising a pair of terminal reverse-complementary fragments each located on one end of the DNA molecule, an expression cassette comprising a coding sequence of the transgene, an anti-sense region that is reverse complementary to the expression cassette, and a central reverse-complementary fragment that connects the expression cassette and anti-sense region; and wherein each of the pair of terminal reverse-complementary fragments and the central reverse-complementary fragment is capable of forming a stem-loop structure. In some embodiments, the self-complementary genome is one described in Section 5.4 (Self-complimentary AAV (scAAV) Genome) of the present disclosure. In some embodiments, the self-complementary genome comprises the sequence set forth in SEQ ID NO: 16. In some embodiments, the self-complementary genome consists of the sequence set forth in SEQ ID NO: 16.
- [0216] In some embodiments, the method for treating glioma comprises administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising a recombinant AAV, wherein the recombinant AAV comprises a self-complementary genome comprising a transgene encoding a NeuroD1 polypeptide and further comprises a AAV capsid protein. In some embodiments, the AAV capsid protein is one described in Section 5.5 (Recombinant scAAV Vectors) of the present disclosure. In some embodiments, the AAV capsid is AAV6.
- [0217] In some embodiments, the method for treating glioma comprises administering to a subject in need thereof a pharmaceutical composition comprising from about 5×10^{11} viral genomes (vg) to about 1×10^{14} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 5×10^{11} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 5.1×10^{11} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises ad-

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the subject a pharmaceutical composition comprising about 8.7×10^{13} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 8.8×10^{13} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 8.9×10^{13} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 9×10^{13} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 9.1×10^{13} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 9.2×10^{13} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 9.3×10^{13} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 9.4×10^{13} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 9.5×10^{13} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 9.6×10^{13} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 9.7×10^{13} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 9.8×10^{13} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 9.9×10^{13} vg of the recombinant AAV. In some embodiments, the method for treating glioma comprises administering to the subject a pharmaceutical composition comprising about 1×10^{14} vg of the recombinant AAV.

[0218] In some embodiments, the subject is administered the pharmaceutical composition comprising about 5×10^{11} viral genome (vg) to about 1×10^{14} vg once. In some embodiments, the subject is administered the pharmaceutical composition comprising about 5×10^{11} vg to about 1×10^{14} vg twice. In some embodiments, the subject is administered the pharmaceutical composition comprising about 5×10^{11} vg to about 1×10^{14} vg at least three times. In some embodiments, the subject is administered the

pharmaceutical composition comprising about 5×10^{11} vg to about 1×10^{14} vg at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten times. In some embodiments, each time the pharmaceutical composition that is administered to the subject once, twice or at least three times comprises about 5×10^{11} vg, about 5.1×10^{11} vg, about 5.2×10^{11} vg, about 5.3×10^{11} vg, about 5.4×10^{11} vg, about 5.5×10^{11} vg, about 5.6×10^{11} vg, about 5.7×10^{11} vg, about 5.8×10^{11} vg, about 5.9×10^{11} vg, about 6×10^{11} vg, about 6.1×10^{11} vg, about 6.2×10^{11} vg, about 6.3×10^{11} vg, about 6.4×10^{11} vg, about 6.5×10^{11} vg, about 6.6×10^{11} vg, about 6.7×10^{11} vg, about 6.8×10^{11} vg, about 6.9×10^{11} vg, about 7.0×10^{11} vg, about 7.1×10^{11} vg, about 7.2×10^{11} vg, about 7.3×10^{11} vg, about 7.4×10^{11} vg, about 7.5×10^{11} vg, about 7.6×10^{11} vg, about 7.7×10^{11} vg, about 7.8×10^{11} vg, about 7.9×10^{11} vg, about 8×10^{11} vg, about 8.1×10^{11} vg, about 8.2×10^{11} vg, about 8.3×10^{11} vg, about 8.4×10^{11} vg, about 8.5×10^{11} vg, about 8.6×10^{11} vg, about 8.7×10^{11} vg, about 8.8×10^{11} vg, about 8.9×10^{11} vg, about 9×10^{11} vg, about 9.1×10^{11} vg, about 9.2×10^{11} vg, about 9.3×10^{11} vg, about 9.4×10^{11} vg, about 9.5×10^{11} vg, about 9.6×10^{11} vg, about 9.7×10^{11} vg, about 9.8×10^{11} vg, about 9.9×10^{11} vg, about 1×10^{12} vg, about 1.1×10^{12} vg, about 1.2×10^{12} vg, about 1.3×10^{12} vg, about 1.4×10^{12} vg, about 1.5×10^{12} vg, about 1.6×10^{12} vg, about 1.7×10^{12} vg, about 1.8×10^{12} vg, about 1.9×10^{12} vg, about 2×10^{12} vg, about 2.1×10^{12} vg, about 2.2×10^{12} vg, about 2.3×10^{12} vg, about 2.4×10^{12} vg, about 2.5×10^{12} vg, about 2.6×10^{12} vg, about 2.7×10^{12} vg, about 2.8×10^{12} vg, about 2.9×10^{12} vg, about 3×10^{12} vg, about 3.1×10^{12} vg, about 3.2×10^{12} vg, about 3.3×10^{12} vg, about 3.4×10^{12} vg, about 3.5×10^{12} vg, about 3.6×10^{12} vg, about 3.7×10^{12} vg, about 3.8×10^{12} vg, about 3.9×10^{12} vg, about 4×10^{12} vg, about 4.1×10^{12} vg, about 4.2×10^{12} vg, about 4.3×10^{12} vg, about 4.4×10^{12} vg, about 4.5×10^{12} vg, about 4.6×10^{12} vg, about 4.7×10^{12} vg, about 4.8×10^{12} vg, about 4.9×10^{12} vg, about 5×10^{12} vg, about 5.1×10^{12} vg, about 5.2×10^{12} vg, about 5.3×10^{12} vg, about 5.4×10^{12} vg, about 5.5×10^{12} vg, about 5.6×10^{12} vg, about 5.7×10^{12} vg, about 5.8×10^{12} vg, about 5.9×10^{12} vg, about 6×10^{12} vg, about 6.1×10^{12} vg, about 6.2×10^{12} vg, about 6.3×10^{12} vg, about 6.4×10^{12} vg, about 6.5×10^{12} vg, about 6.6×10^{12} vg, about 6.7×10^{12} vg, about 6.8×10^{12} vg, about 6.9×10^{12} vg, about 7×10^{12} vg, about 7.1×10^{12} vg, about 7.2×10^{12} vg, about 7.3×10^{12} vg, about 7.4×10^{12} vg, about 7.5×10^{12} vg, about 7.6×10^{12} vg, about 7.7×10^{12} vg, about 7.8×10^{12} vg, about 7.9×10^{12} vg, about 8×10^{12} vg, about 8.1×10^{12} vg, about 8.2×10^{12} vg, about 8.3×10^{12} vg, about 8.4×10^{12} vg, about 8.5×10^{12} vg, about 8.6×10^{12} vg, about 8.7×10^{12} vg, about 8.8×10^{12} vg, about 8.9×10^{12} vg, about 9×10^{12} vg, about 9.1×10^{12} vg, about 9.2×10^{12} vg, about 9.3×10^{12} vg, about 9.4×10^{12} vg, about 9.5×10^{12} vg, about 9.6×10^{12} vg, about 9.7×10^{12} vg, about 9.8×10^{12} vg, about 9.9×10^{12} vg, about

1×10^{13} vg, about 1.1×10^{13} vg, about 1.2×10^{13} vg, about 1.3×10^{13} vg, about 1.4×10^{13} vg, about 1.5×10^{13} vg, about 1.6×10^{13} vg, about 1.7×10^{13} vg, about 1.8×10^{13} vg, about 1.9×10^{13} vg, about 2×10^{13} vg, about 2.1×10^{13} vg, about 2.2×10^{13} vg, about 2.3×10^{13} vg, about 2.4×10^{13} vg, about 2.5×10^{13} vg, about 2.6×10^{13} vg, about 2.7×10^{13} vg, about 2.8×10^{13} vg, about 2.9×10^{13} vg, about 3×10^{13} vg, about 3.1×10^{13} vg, about 3.2×10^{13} vg, about 3.3×10^{13} vg, about 3.4×10^{13} vg, about 3.5×10^{13} vg, about 3.6×10^{13} vg, about 3.7×10^{13} vg, about 3.8×10^{13} vg, about 3.9×10^{13} vg, about 4×10^{13} vg, about 4.1×10^{13} vg, about 4.2×10^{13} vg, about 4.3×10^{13} vg, about 4.4×10^{13} vg, about 4.5×10^{13} vg, about 4.6×10^{13} vg, about 4.7×10^{13} vg, about 4.8×10^{13} vg, about 4.9×10^{13} vg, about 5×10^{13} vg, about 5.1×10^{13} vg, about 5.2×10^{13} vg, about 5.3×10^{13} vg, about 5.4×10^{13} vg, about 5.5×10^{13} vg, about 5.6×10^{13} vg, about 5.7×10^{13} vg, about 5.8×10^{13} vg, about 5.9×10^{13} vg, about 6×10^{13} vg, about 6.1×10^{13} vg, about 6.2×10^{13} vg, about 6.3×10^{13} vg, about 6.4×10^{13} vg, about 6.5×10^{13} vg, about 6.6×10^{13} vg, about 6.7×10^{13} vg, about 6.8×10^{13} vg, about 6.9×10^{13} vg, about 7×10^{13} vg, about 7.1×10^{13} vg, about 7.2×10^{13} vg, about 7.3×10^{13} vg, about 7.4×10^{13} vg, about 7.5×10^{13} vg, about 7.6×10^{13} vg, about 7.7×10^{13} vg, about 7.8×10^{13} vg, about 7.9×10^{13} vg, about 8×10^{13} vg, about 8.1×10^{13} vg, about 8.2×10^{13} vg, about 8.3×10^{13} vg, about 8.4×10^{13} vg, about 8.5×10^{13} vg, about 8.6×10^{13} vg, about 8.7×10^{13} vg, about 8.8×10^{13} vg, about 8.9×10^{13} vg, about 9×10^{13} vg, about 9.1×10^{13} vg, about 9.2×10^{13} vg, about 9.3×10^{13} vg, about 9.4×10^{13} vg, about 9.5×10^{13} vg, about 9.6×10^{13} vg, about 9.7×10^{13} vg, about 9.8×10^{13} vg, about 9.9×10^{13} vg, or about 1×10^{14} vg of the recombinant AAV described herein.

[0219] In some embodiments, the sequential administrations of the pharmaceutical composition is separated by at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, at least 15 days, at least 16 days, at least 17 days, at least 18 days, at least 19 days, at least 20 days, at least 21 days, at least 22 days, at least 23 days, at least 24 days, at least 25 days, at least 26 days, at least 27 days, at least 28 days, at least 29 days, at least 30 days, at least 31 days, at least 32 days, at least 33 days, at least 34 days, at least 35 days, at least 36 days, at least 37 days, at least 38 days, at least 39 days, at least 40 days, at least 41 days, at least 42 days, at least 43 days, at least 44 days, at least 45 days, at least 46 days, at least 47 days, at least 48 days, at least 49 days, at least 50 days, at least 51 days, at least 52 days, at least 53 days, at least 54 days, at least 55 days, at least 56 days, at least 57 days, at least 58 days, at least 59 days, at least 60 days, at least 61 days, at least 62 days, at least 63 days, at least 64 days, at least 65 days, at least 66 days, at least 67 days, at least 68 days, at least 69 days, at least 70 days, at least 71 days, at least 72 days, at least 73 days, at least 74 days, at least 75 days, at

least 76 days, at least 77 days, at least 78 days, at least 79 days, at least 80 days, at least 81 days, at least 82 days, at least 83 days, at least 84 days, at least 85 days, at least 86 days, at least 87 days, at least 88 days, at least 89 days, or at least 90 days.

[0220] In some embodiments, the fluid formulation of the pharmaceutical composition is a solution. In some embodiments, the fluid formulation of the pharmaceutical composition comprises the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL, and further comprises potassium chloride, potassium phosphate monobasic, sodium chloride, sodium phosphate dibasic anhydrous, and a surfactant selected from poloxamer 188, polysorbate 20, or polysorbate 80. In some embodiments, the fluid formulation of the pharmaceutical composition comprises the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL, and further comprises potassium chloride, potassium phosphate monobasic, sodium chloride, sodium phosphate dibasic anhydrous, and poloxamer 188.

[0221] In some embodiments, the fluid formulation of the pharmaceutical composition comprises the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL, and further comprises sodium chloride, sodium phosphate, and poloxamer 188. In specific embodiments, the fluid formulation of the pharmaceutical composition comprises sodium chloride at a concentration of about 180 mM. In some embodiments, the fluid formulation comprises sodium phosphate at a concentration of about 10 mM. In some embodiments, the fluid formulation comprises poloxamer 188 at a concentration of about 0.001% weight/volume (0.01 g/L). In some embodiments, the pH of the pharmaceutical composition is about 7.3.

[0222] In some embodiments, the fluid formulation of the pharmaceutical composition comprises the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL, sodium chloride at a concentration of about 180 mM, sodium phosphate at a concentration of about 10 mM, poloxamer 188 at a concentration of about 0.001% weight/volume (0.01 g/L), and the pH of the pharmaceutical composition is about 7.3.

[0223] In some embodiments, the fluid formulation of the pharmaceutical composition comprises the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL, and further comprises sodium chloride, magnesium chloride, Tris hydrochloride, and poloxamer 188. In some embodiments, the fluid formulation of the pharmaceutical composition comprises sodium chloride at a concentration of about 200 mM. In some embodiments, the fluid formulation of the pharmaceutical composition comprises magnesium chloride at a concentration of about 1 mM. In some embodiments, the fluid formulation of the pharmaceutical com-

position comprises Tris hydrochloride at a concentration of about 20 mM. In some embodiments, the fluid formulation of the pharmaceutical composition comprises poloxamer 188 at a concentration of about 0.005%weight/volume (0.05 g/L) . In some embodiments, and wherein the pH of the pharmaceutical composition is about 8.0.

[0224] In some embodiments, the fluid formulation of the pharmaceutical composition comprises the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL, sodium chloride at a concentration of about 200 mM, magnesium chloride at a concentration of about 1 mM, Tris hydrochloride at a concentration of about 20 mM, poloxamer 188 at a concentration of about 0.005%weight/volume (0.05 g/L) , and wherein the pH of the pharmaceutical composition is about 8.0.

[0225] In some embodiments, the fluid formulation of the pharmaceutical composition comprises the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL, and further comprises sodium chloride, calcium chloride, magnesium chloride, sodium phosphate, and poloxamer 188. In some embodiments, the fluid formulation of the pharmaceutical composition comprises sodium chloride at a concentration of about 150 mM. In some embodiments, the fluid formulation of the pharmaceutical composition comprises calcium chloride at a concentration of about 1.4 mM. In some embodiments, the fluid formulation of the pharmaceutical composition comprises magnesium chloride at a concentration of about 0.8 mM. In some embodiments, the fluid formulation of the pharmaceutical composition comprises sodium phosphate at a concentration of about 1 mM. In some embodiments, the fluid formulation of the pharmaceutical composition comprises poloxamer 188 at a concentration of about 0.001%weight/volume (0.01 g/L) . In some embodiments, the pH of the pharmaceutical composition is about 7.4.

[0226] In some embodiments, the fluid formulation of the pharmaceutical composition comprises the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL, sodium chloride at a concentration of about 150 mM; calcium chloride at a concentration of about 1.4 mM; magnesium chloride at a concentration of about 0.8 mM, sodium phosphate at a concentration of about 1 mM, and poloxamer 188 at a concentration of about 0.001%weight/volume (0.01 g/L) ; and wherein the pH of the pharmaceutical composition is about 7.4.

[0227] In some embodiments, the pharmaceutical composition is administered to the subject intratumorally. In some embodiments, the pharmaceutical composition is administered to the subject by intratumoral injection of a fluid formulation of the pharmaceutical composition. In some embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the pharmaceutical composition

is administered into the surgical cavity. According to the present disclosure, administration of a pharmaceutical composition into a surgical cavity can be performed by, for example, injecting a fluid formulation of the pharmaceutical composition into the tissue immediately surrounding the surgical cavity (e.g., the surgical cavity wall), or injecting a fluid formulation of the pharmaceutical composition into the surgical cavity (i.e., a void space in the surgical cavity). In some embodiments, the pharmaceutical composition is administered to the surgical cavity by injecting a fluid formulation of the pharmaceutical composition into the surgical cavity. In some embodiments, the pharmaceutical composition is administered to the surgical cavity by injecting a fluid formulation of the pharmaceutical composition into the surgical cavity wall.

[0228] In some embodiments, the pharmaceutical composition comprising the recombinant AAV as described herein is administered intratumorally to the subject, wherein the intratumoral administration is performed by injecting a fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL. In some embodiments, the fluid formulation of the pharmaceutical composition administered intratumorally comprises the recombinant AAV at a concentration of about 5×10^{11} vg/mL, about 5.1×10^{11} vg/mL, about 5.2×10^{11} vg/mL, about 5.3×10^{11} vg/mL, about 5.4×10^{11} vg/mL, about 5.5×10^{11} vg/mL, about 5.6×10^{11} vg/mL, about 5.7×10^{11} vg/mL, about 5.8×10^{11} vg/mL, about 5.9×10^{11} vg/mL, about 6×10^{11} vg/mL, about 6.1×10^{11} vg/mL, about 6.2×10^{11} vg/mL, about 6.3×10^{11} vg/mL, about 6.4×10^{11} vg/mL, about 6.5×10^{11} vg/mL, about 6.6×10^{11} vg/mL, about 6.7×10^{11} vg/mL, about 6.8×10^{11} vg/mL, about 6.9×10^{11} vg/mL, about 7×10^{11} vg/mL, about 7.1×10^{11} vg/mL, about 7.2×10^{11} vg/mL, about 7.3×10^{11} vg/mL, about 7.4×10^{11} vg/mL, about 7.5×10^{11} vg/mL, about 7.6×10^{11} vg/mL, about 7.7×10^{11} vg/mL, about 7.8×10^{11} vg/mL, about 7.9×10^{11} vg/mL, about 8×10^{11} vg/mL, about 8.1×10^{11} vg/mL, about 8.2×10^{11} vg/mL, about 8.3×10^{11} vg/mL, about 8.4×10^{11} vg/mL, about 8.5×10^{11} vg/mL, about 8.6×10^{11} vg/mL, about 8.7×10^{11} vg/mL, about 8.8×10^{11} vg/mL, about 8.9×10^{11} vg/mL, about 9×10^{11} vg/mL, about 9.1×10^{11} vg/mL, about 9.2×10^{11} vg/mL, about 9.3×10^{11} vg/mL, about 9.4×10^{11} vg/mL, about 9.5×10^{11} vg/mL, about 9.6×10^{11} vg/mL, about 9.7×10^{11} vg/mL, about 9.8×10^{11} vg/mL, about 9.9×10^{11} vg/mL, about 1×10^{12} vg/mL, about 1.1×10^{12} vg/mL, about 1.2×10^{12} vg/mL, about 1.3×10^{12} vg/mL, about 1.4×10^{12} vg/mL, about 1.5×10^{12} vg/mL, about 1.6×10^{12} vg/mL, about 1.7×10^{12} vg/mL, about 1.8×10^{12} vg/mL, about 1.9×10^{12} vg/mL, about 2×10^{12} vg/mL, about 2.1×10^{12} vg/mL, about 2.2×10^{12} vg/mL, about 2.3×10^{12} vg/mL, about 2.4×10^{12} vg/mL, about 2.5×10^{12} vg/mL, about 2.6×10^{12} vg/mL, about 2.7×10^{12} vg/mL, about 2.8×10^{12} vg/mL, about 2.9×10^{12} vg/mL, about 3×10^{12} vg/mL,

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6.9×10¹³ vg/mL, about 7×10¹³ vg/mL, about 7.1×10¹³ vg/mL, about 7.2×10¹³ vg/mL, about 7.3×10¹³ vg/mL, about 7.4×10¹³ vg/mL, about 7.5×10¹³ vg/mL, about 7.6×10¹³ vg/mL, about 7.7×10¹³ vg/mL, about 7.8×10¹³ vg/mL, about 7.9×10¹³ vg/mL, about 8×10¹³ vg/mL, about 8.1×10¹³ vg/mL, about 8.2×10¹³ vg/mL, about 8.3×10¹³ vg/mL, about 8.4×10¹³ vg/mL, about 8.5×10¹³ vg/mL, about 8.6×10¹³ vg/mL, about 8.7×10¹³ vg/mL, about 8.8×10¹³ vg/mL, about 8.9×10¹³ vg/mL, about 9×10¹³ vg/mL, about 9.1×10¹³ vg/mL, about 9.2×10¹³ vg/mL, about 9.3×10¹³ vg/mL, about 9.4×10¹³ vg/mL, about 9.5×10¹³ vg/mL, about 9.6×10¹³ vg/mL, about 9.7×10¹³ vg/mL, about 9.8×10¹³ vg/mL, about 9.9×10¹³ vg/mL, or about 1×10¹⁴ vg/mL.

[0229] In some embodiments, the fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration in the range of from about 5×10¹¹ vg/mL to about 1×10¹⁴ vg/mL is administered intratumorally once. In some embodiments, the fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration in the range of from about 5×10¹¹ vg/mL to about 1×10¹⁴ vg/mL is administered intratumorally twice. In some embodiments, the fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration in the range of from about 5×10¹¹ vg/mL to about 1×10¹⁴ vg/mL is administered intratumorally for at least three times. In some embodiments, the fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration in the range of from about 5×10¹¹ vg/mL to about 1×10¹⁴ vg/mL is administered intratumorally for at least four, at least five, at least six, at least seven, at least eight, at least nine or at least ten times. In some embodiments, each time the intratumoral injection volume of the fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration in the range of from about 5×10¹¹ vg/mL to about 1×10¹⁴ vg/mL is about 0.3 mL to about 5 mL. In some embodiments, each time the intratumoral injection volume of the fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration in the range of from about 5×10¹¹ vg/mL to about 1×10¹⁴ vg/mL is about 0.3 mL, about 0.4 mL, about 0.5 mL, about 0.6 mL, about 0.7 mL, about 0.8 mL, about 0.9 mL, about 1 mL, about 1.1 mL, about 1.2 mL, about 1.3 mL, about 1.4 mL, about 1.5 mL, about 1.6 mL, about 1.7 mL, about 1.8 mL, about 1.9 mL, about 2 mL, about 2.1 mL, about 2.2 mL, about 2.3 mL, about 2.4 mL, about 2.5 mL, about 2.6 mL, about 2.7 mL, about 2.8 mL, about 2.9 mL, about 3.0 mL, about 3.1 mL, about 3.2 mL, about 3.3 mL, about 3.4 mL, about 3.5 mL, about 3.6 mL, about 3.7 mL, about 3.8 mL, about 3.9 mL, about 4 mL, about 4.1 mL, about 4.2 mL, about 4.3 mL, about 4.4 mL, about 4.5 mL, about 4.6 mL, about 4.7 mL, about 4.8 mL, about 4.9 mL, or about 5 mL. In specific embodiments,

for at least one of the sequential administrations, 1mL of a fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration of about 2×10^{12} vg/mL is injected intratumorally. In specific embodiments, for at least one of the sequential administrations, 1mL of a fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration of about 6.6×10^{12} vg/mL is injected intratumorally. In specific embodiments, for at least one of the sequential intratumoral injections, 1mL of a fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration of about 2×10^{13} vg/mL is injected intratumorally. In some embodiments, sequential administrations of the fluid formulation of the pharmaceutical composition is separated by at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, at least 15 days, at least 16 days, at least 17 days, at least 18 days, at least 19 days, at least 20 days, at least 21 days, at least 22 days, at least 23 days, at least 24 days, at least 25 days, at least 26 days, at least 27 days, at least 28 days, at least 29 days, at least 30 days, at least 31 days, at least 32 days, at least 33 days, at least 34 days, at least 35 days, at least 36 days, at least 37 days, at least 38 days, at least 39 days, at least 40 days, at least 41 days, at least 42 days, at least 43 days, at least 44 days, at least 45 days, at least 46 days, at least 47 days, at least 48 days, at least 49 days, at least 50 days, at least 51 days, at least 52 days, at least 53 days, at least 54 days, at least 55 days, at least 56 days, at least 57 days, at least 58 days, at least 59 days, at least 60 days, at least 61 days, at least 62 days, at least 63 days, at least 64 days, at least 65 days, at least 66 days, at least 67 days, at least 68 days, at least 69 days, at least 70 days, at least 71 days, at least 72 days, at least 73 days, at least 74 days, at least 75 days, at least 76 days, at least 77 days, at least 78 days, at least 79 days, at least 80 days, at least 81 days, at least 82 days, at least 83 days, at least 84 days, at least 85 days, at least 86 days, at least 87 days, at least 88 days, at least 89 days, or at least 90 days.

[0230] In some embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the pharmaceutical composition is administered into the surgical cavity. In some embodiments, the intracavity administration is by injecting a fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL. In some embodiments, the fluid formulation of the pharmaceutical composition administered into the surgical cavity comprises the recombinant AAV at a concentration of about 5×10^{11} vg/mL, about 5.1×10^{11} vg/mL, about 5.2×10^{11} vg/mL, about 5.3×10^{11} vg/mL, about 5.4×10^{11} vg/mL, about 5.5×10^{11} vg/mL, about 5.6×10^{11} vg/mL, about 5.7×10^{11} vg/mL, about 5.8×10^{11} vg/mL, about 5.9×10^{11} vg/mL, about 6×10^{11} vg/mL,

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9.9×10^{12} vg/mL, about 1×10^{13} vg/mL, about 1.1×10^{13} vg/mL, about 1.2×10^{13} vg/mL, about 1.3×10^{13} vg/mL, about 1.4×10^{13} vg/mL, about 1.5×10^{13} vg/mL, about 1.6×10^{13} vg/mL, about 1.7×10^{13} vg/mL, about 1.8×10^{13} vg/mL, about 1.9×10^{13} vg/mL, about 2×10^{13} vg/mL, about 2.1×10^{13} vg/mL, about 2.2×10^{13} vg/mL, about 2.3×10^{13} vg/mL, about 2.4×10^{13} vg/mL, about 2.5×10^{13} vg/mL, about 2.6×10^{13} vg/mL, about 2.7×10^{13} vg/mL, about 2.8×10^{13} vg/mL, about 2.9×10^{13} vg/mL, about 3×10^{13} vg/mL, about 3.1×10^{13} vg/mL, about 3.2×10^{13} vg/mL, about 3.3×10^{13} vg/mL, about 3.4×10^{13} vg/mL, about 3.5×10^{13} vg/mL, about 3.6×10^{13} vg/mL, about 3.7×10^{13} vg/mL, about 3.8×10^{13} vg/mL, about 3.9×10^{13} vg/mL, about 4×10^{13} vg/mL, about 4.1×10^{13} vg/mL, about 4.2×10^{13} vg/mL, about 4.3×10^{13} vg/mL, about 4.4×10^{13} vg/mL, about 4.5×10^{13} vg/mL, about 4.6×10^{13} vg/mL, about 4.7×10^{13} vg/mL, about 4.8×10^{13} vg/mL, about 4.9×10^{13} vg/mL, about 5×10^{13} vg/mL, about 5.1×10^{13} vg/mL, about 5.2×10^{13} vg/mL, about 5.3×10^{13} vg/mL, about 5.4×10^{13} vg/mL, about 5.5×10^{13} vg/mL, about 5.6×10^{13} vg/mL, about 5.7×10^{13} vg/mL, about 5.8×10^{13} vg/mL, about 5.9×10^{13} vg/mL, about 6×10^{13} vg/mL, about 6.1×10^{13} vg/mL, about 6.2×10^{13} vg/mL, about 6.3×10^{13} vg/mL, about 6.4×10^{13} vg/mL, about 6.5×10^{13} vg/mL, about 6.6×10^{13} vg/mL, about 6.7×10^{13} vg/mL, about 6.8×10^{13} vg/mL, about 6.9×10^{13} vg/mL, about 7×10^{13} vg/mL, about 7.1×10^{13} vg/mL, about 7.2×10^{13} vg/mL, about 7.3×10^{13} vg/mL, about 7.4×10^{13} vg/mL, about 7.5×10^{13} vg/mL, about 7.6×10^{13} vg/mL, about 7.7×10^{13} vg/mL, about 7.8×10^{13} vg/mL, about 7.9×10^{13} vg/mL, about 8×10^{13} vg/mL, about 8.1×10^{13} vg/mL, about 8.2×10^{13} vg/mL, about 8.3×10^{13} vg/mL, about 8.4×10^{13} vg/mL, about 8.5×10^{13} vg/mL, about 8.6×10^{13} vg/mL, about 8.7×10^{13} vg/mL, about 8.8×10^{13} vg/mL, about 8.9×10^{13} vg/mL, about 9×10^{13} vg/mL, about 9.1×10^{13} vg/mL, about 9.2×10^{13} vg/mL, about 9.3×10^{13} vg/mL, about 9.4×10^{13} vg/mL, about 9.5×10^{13} vg/mL, about 9.6×10^{13} vg/mL, about 9.7×10^{13} vg/mL, about 9.8×10^{13} vg/mL, about 9.9×10^{13} vg/mL, or about 1×10^{14} vg/mL.

[0231] In some embodiments, the fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL is administered into the surgical cavity once. In some embodiments, the fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL is administered into the surgical cavity twice. In some embodiments, the fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL is administered into the surgical cavity at least three times. In some embodiments, the fluid formulation of the pharmaceutical composition comprising the recombinant

AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL is administered into the surgical cavity at least four, at least five, at least six, at least seven, at least eight, at least nine or at least ten times. In some embodiments, intracavity administration is performed immediately following the surgical removal of a tumor (e.g., a glioma) from the subject. In specific embodiments, the first one of the sequential administrations is performed by injecting a fluid formulation of the pharmaceutical composition comprising the recombinant AAV at the concentration of about 5×10^{11} vg/mL to about 1×10^{14} vg/mL into the surgical cavity wall, and the following one or more of the sequential administrations is (are) performed by injecting a fluid formulation of the pharmaceutical composition comprising the recombinant AAV at the concentration of about 5×10^{11} vg/mL to about 1×10^{14} vg/mL into a void space in the surgical cavity. In some embodiments, each time the intracavity administration volume of the fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL is about 0.3 mL to about 5 mL. In some embodiments, each time the intracavity administration volume of the fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration in the range of from about 5×10^{11} vg/mL to about 1×10^{14} vg/mL is about 0.3 mL, about 0.4 mL, about 0.5 mL, about 0.6 mL, about 0.7 mL, about 0.8 mL, about 0.9 mL, about 1 mL, about 1.1 mL, about 1.2 mL, about 1.3 mL, about 1.4 mL, about 1.5 mL, about 1.6 mL, about 1.7 mL, about 1.8 mL, about 1.9 mL, about 2 mL, about 2.1 mL, about 2.2 mL, about 2.3 mL, about 2.4 mL, about 2.5 mL, about 2.6 mL, about 2.7 mL, about 2.8 mL, about 2.9 mL, about 3.0 mL, about 3.1 mL, about 3.2 mL, about 3.3 mL, about 3.4 mL, about 3.5 mL, about 3.6 mL, about 3.7 mL, about 3.8 mL, about 3.9 mL, about 4 mL, about 4.1 mL, about 4.2 mL, about 4.3 mL, about 4.4 mL, about 4.5 mL, about 4.6 mL, about 4.7 mL, about 4.8 mL, about 4.9 mL, or about 5 mL. In specific embodiments, for at least one of the sequential administrations, 2mL of a fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration of about 2×10^{12} vg/mL is administered into a surgical cavity. In specific embodiments, for at least one of the sequential administrations, 2mL of a fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration of about 6.6×10^{12} vg/mL is administered into a surgical cavity. In specific embodiments, for at least one of the sequential administrations, 2mL of a fluid formulation of the pharmaceutical composition comprising the recombinant AAV at a concentration of about 2×10^{13} vg/mL is administered into a surgical cavity. In some embodiments, sequential administration of the fluid formulation of the pharmaceutical composition is separated by at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days,

at least 13 days, at least 14 days, at least 15 days, at least 16 days, at least 17 days, at least 18 days, at least 19 days, at least 20 days, at least 21 days, at least 22 days, at least 23 days, at least 24 days, at least 25 days, at least 26 days, at least 27 days, at least 28 days, at least 29 days, at least 30 days, at least 31 days, at least 32 days, at least 33 days, at least 34 days, at least 35 days, at least 36 days, at least 37 days, at least 38 days, at least 39 days, at least 40 days, at least 41 days, at least 42 days, at least 43 days, at least 44 days, at least 45 days, at least 46 days, at least 47 days, at least 48 days, at least 49 days, at least 50 days, at least 51 days, at least 52 days, at least 53 days, at least 54 days, at least 55 days, at least 56 days, at least 57 days, at least 58 days, at least 59 days, or at least 60 days, at least 61 days, at least 62 days, at least 63 days, at least 64 days, at least 65 days, at least 66 days, at least 67 days, at least 68 days, at least 69 days, at least 70 days, at least 71 days, at least 72 days, at least 73 days, at least 74 days, at least 75 days, at least 76 days, at least 77 days, at least 78 days, at least 79 days, at least 80 days, at least 81 days, at least 82 days, at least 83 days, at least 84 days, at least 85 days, at least 86 days, at least 87 days, at least 88 days, at least 89 days, or at least 90 days.

[0232] In specific embodiments, the subject is administered intratumorally the pharmaceutical composition comprising about 2×10^{12} vg of the recombinant AAV once. In specific embodiments, the subject receives intratumoral injection of 1 mL of the pharmaceutical composition comprising about 2×10^{12} vg/mL of the recombinant AAV. In some embodiments, the pharmaceutical composition is administered intratumorally through stereotactic brain injection. In some embodiments, the pharmaceutical composition is administered intratumorally through an Ommaya reservoir.

[0233] In specific embodiments, the subject is administered intratumorally the pharmaceutical composition comprising about 2×10^{12} vg of the recombinant AAV once every 15 days twice. In specific embodiments, each time the subject receives intratumoral injection of 1 mL of the pharmaceutical composition comprising about 2×10^{12} vg/mL of the recombinant AAV. In some embodiments, each time the pharmaceutical composition is administered intratumorally through stereotactic brain injection. In some embodiments, each time the pharmaceutical composition is administered intratumorally through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered intratumorally through stereotactic brain injection, and for the second time the pharmaceutical composition is administered intratumorally through an Ommaya reservoir.

[0234] In specific embodiments, the subject is administered intratumorally the pharmaceutical composition comprising about 2×10^{12} vg of the recombinant AAV once every 15 days for three times. In specific embodiments, each time the subject receives in-

tratumoral injection of 1 mL of the pharmaceutical composition comprising about 2×10^{12} vg/mL of the recombinant AAV. In some embodiments, each time the pharmaceutical composition is administered intratumorally through stereotactic brain injection. In some embodiments, each time the pharmaceutical composition is administered intratumorally through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered intratumorally through stereotactic brain injection, and for the second and subsequent times the pharmaceutical composition is administered intratumorally through an Ommaya reservoir.

[0235] In specific embodiments, the subject is administered intratumorally the pharmaceutical composition comprising about 2×10^{12} vg of the recombinant AAV once every 15 days for more than three times. In specific embodiments, each time the subject receives intratumoral injection of 1 mL of the pharmaceutical composition comprising about 2×10^{12} vg/mL of the recombinant AAV. In some embodiments, each time the pharmaceutical composition is administered intratumorally through stereotactic brain injection. In some embodiments, each time the pharmaceutical composition is administered intratumorally through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered intratumorally through stereotactic brain injection, and for the second and subsequent times the pharmaceutical composition is administered intratumorally through an Ommaya reservoir.

[0236] In specific embodiments, the subject is administered intratumorally the pharmaceutical composition comprising about 6.6×10^{12} vg of the recombinant AAV once. In specific embodiments, the subject receives intratumoral injection of 1 mL of the pharmaceutical composition comprising about 6.6×10^{12} vg/mL of the recombinant AAV. In some embodiments, the pharmaceutical composition is administered intratumorally through stereotactic brain injection. In some embodiments, the pharmaceutical composition is administered intratumorally through an Ommaya reservoir.

[0237] In specific embodiments, the subject is administered intratumorally the pharmaceutical composition comprising about 6.6×10^{12} vg of the recombinant AAV once every 15 days twice. In specific embodiments, each time the subject receives intratumoral injection of 1 mL of the pharmaceutical composition comprising about 6.6×10^{12} vg/mL of the recombinant AAV. In some embodiments, each time the pharmaceutical composition is administered intratumorally through stereotactic brain injection. In some embodiments, each time the pharmaceutical composition is administered intratumorally through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered intratumorally through stereotactic brain injection, and for the second time the pharmaceutical composition is administered intratumorally through an Ommaya reservoir.

- [0238] In specific embodiments, the subject is administered intratumorally the pharmaceutical composition comprising about 6.6×10^{12} vg of the recombinant AAV once every 15 days for three times. In specific embodiments, each time the subject receives intratumoral injection of 1 mL of the pharmaceutical composition comprising about 6.6×10^{12} vg/mL of the recombinant AAV. In some embodiments, each time the pharmaceutical composition is administered intratumorally through stereotactic brain injection. In some embodiments, each time the pharmaceutical composition is administered intratumorally through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered intratumorally through stereotactic brain injection, and for the second and subsequent times the pharmaceutical composition is administered intratumorally through an Ommaya reservoir.
- [0239] In specific embodiments, the subject is administered intratumorally the pharmaceutical composition comprising about 6.6×10^{12} vg of the recombinant AAV once every 15 days for more than three times. In specific embodiments, each time the subject receives intratumoral injection of 1 mL of the pharmaceutical composition comprising about 6.6×10^{12} vg/mL of the recombinant AAV. In some embodiments, each time the pharmaceutical composition is administered intratumorally through stereotactic brain injection. In some embodiments, each time the pharmaceutical composition is administered intratumorally through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered intratumorally through stereotactic brain injection, and for the second and subsequent times the pharmaceutical composition is administered intratumorally through an Ommaya reservoir.
- [0240] In specific embodiments, the subject is administered intratumorally the pharmaceutical composition comprising about 2×10^{13} vg of the recombinant AAV once. In specific embodiments, the subject receives intratumoral injection of 1 mL of the pharmaceutical composition comprising about 2×10^{13} vg/mL of the recombinant AAV. In some embodiments, the pharmaceutical composition is administered intratumorally through stereotactic brain injection. In some embodiments, the pharmaceutical composition is administered intratumorally through an Ommaya reservoir.
- [0241] In specific embodiments, the subject is administered intratumorally the pharmaceutical composition comprising about 2×10^{13} vg of the recombinant AAV once every 15 days twice. In specific embodiments, each time the subject receives intratumoral injection of 1 mL of the pharmaceutical composition comprising about 2×10^{13} vg/mL of the recombinant AAV. In some embodiments, each time the pharmaceutical composition is administered intratumorally through stereotactic brain injection. In some embodiments, each time the pharmaceutical composition is administered intra-

tumorally through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered intratumorally through stereotactic brain injection, and for the second time the pharmaceutical composition is administered intratumorally through an Ommaya reservoir.

[0242] In specific embodiments, the subject is administered intratumorally the pharmaceutical composition comprising about 2×10^{13} vg of the recombinant AAV once every 15 days for three times. In specific embodiments, each time the subject receives intratumoral injection of 1 mL of the pharmaceutical composition comprising about 2×10^{13} vg/mL of the recombinant AAV. In some embodiments, each time the pharmaceutical composition is administered intratumorally through stereotactic brain injection. In some embodiments, each time the pharmaceutical composition is administered intratumorally through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered intratumorally through stereotactic brain injection, and for the second and subsequent times the pharmaceutical composition is administered intratumorally through an Ommaya reservoir.

[0243] In specific embodiments, the subject is administered intratumorally the pharmaceutical composition comprising about 2×10^{13} vg of the recombinant AAV once every 15 days for more than three times. In specific embodiments, each time the subject receives intratumoral injection of 1 mL of the pharmaceutical composition comprising about 2×10^{13} vg/mL of the recombinant AAV. In some embodiments, each time the pharmaceutical composition is administered intratumorally through stereotactic brain injection. In some embodiments, each time the pharmaceutical composition is administered intratumorally through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered intratumorally through stereotactic brain injection, and for the second and subsequent times the pharmaceutical composition is administered intratumorally through an Ommaya reservoir.

[0244] In specific embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 4×10^{12} vg of the recombinant AAV to the surgical cavity once. In specific embodiments, the subject receives intratumoral injection of 2 mL of the pharmaceutical composition comprising about 2×10^{12} vg/mL of the recombinant AAV. In some embodiments, the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma. In some embodiments, the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir.

[0245] In specific embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical com-

position comprising about 4×10^{12} vg of the recombinant AAV to the surgical cavity once every 30 days for twice. In specific embodiments, each time the subject receives administration of 2 mL of the pharmaceutical composition comprising about 2×10^{12} vg/mL of the recombinant AAV to the surgical cavity. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma, and for the second time, the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir.

[0246] In specific embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 4×10^{12} vg of the recombinant AAV to the surgical cavity for once every 30 days for three times. In specific embodiments, each time the subject receives administration of 2 mL of the pharmaceutical composition comprising about 2×10^{12} vg/mL of the recombinant AAV to the surgical cavity. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma, and for the second and subsequent times, the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir.

[0247] In specific embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 4×10^{12} vg of the recombinant AAV to the surgical cavity once every 30 days for more than three times. In specific embodiments, each time the subject receives administration of 2 mL of the pharmaceutical composition comprising about 2×10^{12} vg/mL of the recombinant AAV to the surgical cavity. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma, and for the second and subsequent times, the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir.

- [0248] In specific embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 1.32×10^{13} vg of the recombinant AAV to the surgical cavity once. In specific embodiments, the subject receives intratumoral injection of 2 mL of the pharmaceutical composition comprising about 6.6×10^{12} vg/mL of the recombinant AAV. In some embodiments, the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma. In some embodiments, the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir.
- [0249] In specific embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 1.32×10^{13} of the recombinant AAV to the surgical cavity once every 30 days for twice. In specific embodiments, each time the subject receives administration of 2 mL of the pharmaceutical composition comprising about 6.6×10^{12} vg/mL of the recombinant AAV to the surgical cavity. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma, and for the second time, the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir.
- [0250] In specific embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 1.32×10^{13} vg of the recombinant AAV to the surgical cavity for once every 30 days for three times. In specific embodiments, each time the subject receives administration of 2 mL of the pharmaceutical composition comprising about 6.6×10^{12} vg/mL of the recombinant AAV to the surgical cavity. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma, and for the second and subsequent times, the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir.
- [0251] In specific embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical com-

position comprising about 1.32×10^{13} vg of the recombinant AAV to the surgical cavity once every 30 days for more than three times. In specific embodiments, each time the subject receives administration of 2 mL of the pharmaceutical composition comprising about 6.6×10^{12} vg/mL of the recombinant AAV to the surgical cavity. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma, and for the second and subsequent times, the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir.

[0252] In specific embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 4×10^{13} vg of the recombinant AAV to the surgical cavity once. In specific embodiments, the subject receives intratumoral injection of 2 mL of the pharmaceutical composition comprising about 2×10^{13} vg/mL of the recombinant AAV. In some embodiments, the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma. In some embodiments, the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir.

[0253] In specific embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 4×10^{13} of the recombinant AAV to the surgical cavity once every 30 days for twice. In specific embodiments, each time the subject receives administration of 2 mL of the pharmaceutical composition comprising about 2×10^{13} vg/mL of the recombinant AAV to the surgical cavity. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma, and for the second time, the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir.

[0254] In specific embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 4×10^{13} vg of the recombinant AAV to the surgical cavity for once every 30 days for three times. In specific embodiments, each time the subject

receives administration of 2 mL of the pharmaceutical composition comprising about 2×10^{13} vg/mL of the recombinant AAV to the surgical cavity. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma, and for the second and subsequent times, the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir.

[0255] In specific embodiments, the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 4×10^{13} vg of the recombinant AAV to the surgical cavity once every 30 days for more than three times. In specific embodiments, each time the subject receives administration of 2 mL of the pharmaceutical composition comprising about 2×10^{13} vg/mL of the recombinant AAV to the surgical cavity. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma. In some embodiments, each time the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir. In some embodiments, for the first time the pharmaceutical composition is administered into the surgical cavity immediately after surgically removing the glioma, and for the second and subsequent times, the pharmaceutical composition is administered into the surgical cavity through an Ommaya reservoir.

[0256] In some embodiments, upon administration of the recombinant AAV encoding a NeuroD1 polypeptide as described herein, or a pharmaceutical composition thereof, to a subject suffering from glioma, the NeuroD1 polypeptide encoded by the recombinant AAV genome is expressed in a population of glioma cells in the subject.

[0257] In some embodiments, upon administration of the recombinant AAV encoding a NeuroD1 polypeptide as described herein, or a pharmaceutical composition thereof, to the subject suffering from glioma, the NeuroD1 polypeptide encoded by the recombinant AAV genome is expressed in a population of glioma cells in the subject, and the population of glioma cells stops proliferation in the subject. In specific embodiments, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95% of the glioma cells in the population stops proliferation. In some embodiments, the proliferative activity in a population of glioma cells is measure by the expression level of nuclear protein markers, such as Ki67. In specific embodiments, reduction in cell proliferation occurs in less than about 21 days,

less than about 14 days, less than about 13 days, less than about 12 days, less than about 11 days, less than about 10 days, less than about 9 days, less than about 8 days, less than about 7 days, less than about 6 days, less than about 5 days, less than about 4 days, less than about 3 days, less than about 2 days, or less than about 1 day after the subject received administration of the recombinant AAV encoding a NeuroD1 polypeptide as described herein, or a pharmaceutical composition thereof.

[0258] In some embodiments, upon administration of the recombinant AAV encoding a NeuroD1 polypeptide as described herein, or a pharmaceutical composition thereof, to the subject suffering from glioma, the NeuroD1 polypeptide encoded by the recombinant AAV genome is expressed in a population of glioma cells in the subject, and the population of glioma cells start to exhibit one or more neuronal phenotypes. In some embodiments, the one or more neuronal phenotypes comprise expression of one or more neuronal markers selected from DCX, TUJ1, NeuN, and MAP2. In some embodiments, the one or more neuronal phenotypes comprise ability of firing action potentials. In some embodiments, the one or more neuronal phenotypes comprise formation of dendrites and/or axons on the cell surface. In some embodiments, the one or more neuronal phenotypes comprise formation of synapses with a neighboring cell. In some embodiments, the one or more neuronal phenotypes comprise the ability of releasing synaptic currents. In some embodiments, the synaptic currents are glutamatergic current, GABAergic current, Dopaminergic current, glycinergic current, serotonergic current or norepinephrinergic current. In specific embodiments, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95% of the glioma cells in the population start to exhibit one or more neuronal phenotypes. In specific embodiments, the population of glioma cells start to exhibit one or more neuronal phenotypes in less than about 21 days, less than about 14 days, less than about 13 days, less than about 12 days, less than about 11 days, less than about 10 days, less than about 9 days, less than about 8 days, less than about 7 days, less than about 6 days, less than about 5 days, less than about 4 days, less than about 3 days, less than about 2 days, or less than about 1 day after the subject received administration of the recombinant AAV encoding a NeuroD1 polypeptide as described herein, or a pharmaceutical composition thereof.

[0259] In some embodiments, upon administration of the recombinant AAV encoding a NeuroD1 polypeptide as described herein, or a pharmaceutical composition thereof, to the subject suffering from glioma, the NeuroD1 polypeptide encoded by the recombinant AAV genome is expressed in a population of glioma cells in the subject, and the population of glioma cells stop expressing one or more glial markers. In some embodiments, the one or more glial markers is selected from GFAP, Aldh1l1,

S100 β and Sox9. In specific embodiments, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95% of the glioma cells in the population stop to express one or more glial markers. In specific embodiments, the population of glioma cells stop to express one or more glial markers in less than about 21 days, less than about 14 days, less than about 13 days, less than about 12 days, less than about 11 days, less than about 10 days, less than about 9 days, less than about 8 days, less than about 7 days, less than about 6 days, less than about 5 days, less than about 4 days, less than about 3 days, less than about 2 days, or less than about 1 day after the subject received administration of the recombinant AAV encoding a NeuroD1 polypeptide as described herein, or a pharmaceutical composition thereof.

[0260] In some embodiments, upon administration of the recombinant AAV encoding a NeuroD1 polypeptide as described herein, or a pharmaceutical composition thereof, to the subject suffering from glioma, the NeuroD1 polypeptide encoded by the recombinant AAV genome is expressed in a population of glioma cells in the subject, and the population of glioma cells trans-differentiate into neurons. In some embodiments, the neurons are selected from glutamatergic neurons, GABAergic neurons, dopaminergic neurons; motor neurons, glycinergic neurons, serotonergic neurons, nor-epinephrinergic neurons, and sensory neurons. In specific embodiments, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95% of the glioma cells in the population transdifferentiate into neurons. In specific embodiments, the population of glioma cells transdifferentiate into neurons in less than about 21 days, less than about 14 days, less than about 13 days, less than about 12 days, less than about 11 days, less than about 10 days, less than about 9 days, less than about 8 days, less than about 7 days, less than about 6 days, less than about 5 days, less than about 4 days, less than about 3 days, less than about 2 days, or less than about 1 day after the subject received administration of the recombinant AAV encoding a NeuroD1 polypeptide as described herein, or a pharmaceutical composition thereof.

[0261] In some embodiments, upon administration of the recombinant AAV encoding a NeuroD1 polypeptide as described herein, or a pharmaceutical composition thereof, to the subject suffering from glioma, the NeuroD1 polypeptide encoded by the recombinant AAV genome is expressed in a population of glioma cells in the subject, and the population of glioma cells undergo apoptosis. In specific embodiments, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about

90%, or at least about 95% of the glioma cells in the population undergo apoptosis. In specific embodiments, the population of glioma cells start to undergo apoptosis in less than 21 days, less than 14 days, less than 13 days, less than 12 days, less than 11 days, less than 10 days, less than 9 days, less than 8 days, less than 7 days, less than 6 days, less than 5 days, less than 4 days, less than 3 days, less than 2 days, or less than 1 day after the subject received administration of the recombinant AAV encoding a NeuroD1 polypeptide as described herein, or a pharmaceutical composition thereof.

[0262] In some embodiments, the population of glioma cells are located in the brain of a subject. In some embodiments, the population of glioma cells are located in the grey matter of the brain. In some embodiments, the population of glioma cells are located in the white matter of the brain. In some embodiments, the population of glioma cells are located in the brain striatum. In some embodiments, the population of glioma cells are located in the cortex of the brain. In some embodiments, the population of glioma cells are located in the hippocampus of the brain. In some embodiments, the population of glioma cells are located in the cerebellum of the brain. In some embodiments, the population of glioma cells comprises one or more glial cell types selected from astrocytes, reactive astrocytes, NG-2 cells, reactive NG-2 cells, and microglial cells that are undergoing pathogenic neoplasm. In some embodiments, the population of glioma cells form one or more gliomas in the tissue where they locate.

[0263] In some embodiments, upon administration of the recombinant AAV encoding a NeuroD1 polypeptide as described herein, or a pharmaceutical composition thereof, to the subject suffering from glioma, the NeuroD1 polypeptide encoded by the recombinant AAV genome is expressed in a population of glioma cells in the subject, and the size of the glioma containing the population of glioma cells is reduced. In specific embodiments, the size of the glioma is reduced for at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95%. In some embodiments, the life span of the subject suffering from glioma is increased. In some embodiments, the life span of the subject suffering from glioma is increased for at least 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95%.

[0264] In some embodiments, upon administration of the recombinant AAV encoding a NeuroD1 polypeptide as described herein, or a pharmaceutical composition thereof, to the subject, innate immune response is upregulated in the tissue area receiving the administration. In some embodiments, innate immune response level is measured by upregulation of Iba1 expression. In some embodiments, Iba1 expression in the tissue area that receiving administration of the recombinant AAV encoding a NeuroD1

polypeptide as described herein, or a pharmaceutical composition thereof, is increased for at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95%.

- [0265] In certain embodiments, the glioma is selected from adult-type diffuse gliomas; astrocytoma, IDH-mutant; oligodendroglioma, IDH-mutant, and 1p/19q-codeleted; glioblastoma, IDH-wildtype; pediatric-type diffuse low-grade gliomas; diffuse astrocytoma, MYB-or MYBL1-altered; angiocentric glioma; polymorphous low-grade neuroepithelial tumor of the young; diffuse low-grade glioma, MAPK pathway-altered; pediatric-type diffuse high-grade gliomas; diffuse midline glioma, H3 K27-altered; diffuse hemispheric glioma, H3 G34-mutant; diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype; infant-type hemispheric glioma; circumscribed astrocytic gliomas; pilocytic astrocytoma; high-grade astrocytoma with piloid features; pleomorphic xanthoastrocytoma; subependymal giant cell astrocytoma; chordoid glioma; astroblastoma, MN1-altered; ganglioglioma; and esmoplastic infantile ganglioglioma. In certain embodiments, the glioma is classified Grade 1, Grade 2, Grade 3 and/or Grade 4 according to the World Health Organization (WHO) classification of nervous system tumors as reported in Louis et al., The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro Oncol* 23, 1231-1251 (2021) the content of which is incorporated by reference in its entirety.
- [0266] In specific embodiments, the glioma is selected from oligodendroglioma, IDH mutant, and 1p/19q-codeleted (WHO grade 3) ; astrocytoma, IDH mutant (WHO grade 3, 4) , and glioblastoma, IDH wildtype (WHO grade 4) . In specific embodiments, the glioma is selected from diffuse midline glioma, H3 K27-altered; diffuse hemispheric glioma, H3 G34-mutant; diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype; and infant-type hemispheric glioma.
- [0267] In certain embodiments, the subject is a mammal. In certain embodiments, the subject is a primate (e.g., a monkey such as, a cynomolgus monkey and a human) or a non-primate (e.g., a cow, a pig, a horse, a cat, a dog, a rat, a mouse) . In certain embodiments, the subject is a mouse or a rat. In certain embodiments, the subject is a human.
- [0268] In some embodiments, the subject is treatment^{naïve}. In some embodiments, the subject suffering from glioma has been treated previously for glioma before receiving the treatment of a recombinant AAV encoding NeuroD1 as described herein. In some embodiments, the subject is refractory to the previous treatment. In some embodiments, the glioma is relapsed from the previous treatment.
- [0269] In some embodiments, the previous therapy is a chemotherapeutic agent and the cancer is refractory or relapsed from such previous treatment. In specific embodiments,

the previous therapy is treatment with a chemotherapeutic agent selected from temozolomide (Temozolomide[®]), Carmustine (BCNU), and Lomustine (CCNU).

- [0270] In some embodiments, the previous therapy is a radiation therapy, and the cancer is refractory or relapsed from such previous treatment. In some embodiments, the radiation therapy comprises the use of x-rays, gamma rays and other sources of radiation to destroy cancer cells.
- [0271] In some embodiments, the previous therapy is a hormonal agent and the cancer is refractory or relapsed from such previous treatment. In some embodiments, the hormonal agent is glucocorticoid.
- [0272] In certain embodiments, the recombinant AAV vector may be administered alone or in combination with other prophylactic and/or therapeutic agents. In certain embodiments, the presently disclosed AAV vectors are administered intravenously and may be administered together with other biologically active agents.
- [0273] The dosage amounts and frequencies of administration provided herein are encompassed by the terms therapeutically effective and prophylactically effective. The dosage and frequency typically vary according to factors specific for each patient depending on the specific therapeutic or prophylactic AAV vectors administered, the severity and type of disease, the route of administration, as well as age, body weight, response, and the past medical history of the patient, and should be decided according to the judgment of the practitioner and each patient's circumstances. Suitable regimens can be selected by one skilled in the art by considering such factors and by following, for example, dosages reported in the literature and recommended in the Physician's Desk Reference. Prophylactic and/or therapeutic AAV vectors can be administered repeatedly. Several aspects of the procedure may vary such as the temporal regimen of administering the prophylactic or therapeutic AAV vectors, and whether such AAV vectors are administered separately or as an admixture.
- [0274] Effective doses of the AAV vector can be determined by standard clinical techniques. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems. In certain embodiments, the therapeutically effective dose can be estimated initially from cell culture assays.
- [0275] The presently disclosed AAV vectors, as well as combinations thereof, can be tested in suitable animal model systems prior to use in humans. Such animal model systems include, but are not limited to, rats, mice, chicken, cows, monkeys, pigs, dogs, rabbits, etc. Any animal system known in the art may be used. Such model systems are widely used and well known to the skilled artisan. In certain embodiments, animal model systems for a CNS condition are used that are based on rats, mice, or other small mammal other than a primate.

- [0276] Once the presently disclosed AAV vectors have been tested in an animal model, they can be tested in clinical trials to establish their efficacy. Establishing clinical trials will be done in accordance with common methodologies known to one skilled in the art, and the optimal dosages and routes of administration as well as toxicity profiles of the presently disclosed AAV vectors can be established. For example, a clinical trial can be designed to test the presently disclosed AAV vectors for efficacy and toxicity in human patients.
- [0277] Toxicity and efficacy of the presently disclosed AAV vectors can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. AAV vectors that exhibit large therapeutic indices are preferred. While AAV vectors that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such AAV vectors to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.
- [0278] The presently disclosed AAV vectors generally will be administered for a time and in an amount effective for obtain a desired therapeutic and/or prophylactic benefit. The data obtained from the cell culture assays and animal studies can be used in formulating a range and/or schedule for dosage of the presently disclosed AAV vectors for use in humans. The dosage of such AAV vectors lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.
- [0279] In certain embodiment, the presently disclosed AAV vectors may be delivered in a sustained release formulation, e.g., where the formulations provide extended release and thus extended half-life of the administered AAV vectors. Controlled release systems suitable for use include, without limitation, diffusion-controlled, solvent-controlled, and chemically-controlled systems. Diffusion controlled systems include, for example reservoir devices, in which the molecules of the invention are enclosed within a device such that release of the molecules is controlled by permeation through a diffusion barrier. Common reservoir devices include, for example, membranes, capsules, microcapsules, liposomes, and hollow fibers. Monolithic (matrix) devices are a second type of diffusion-controlled system, wherein the dual antigen-binding molecules are dispersed or dissolved in a rate-controlling matrix (e.g., a polymer matrix). The AAV vectors can be homogeneously dispersed throughout a rate-controlling matrix and the rate of release is controlled by diffusion through the matrix.

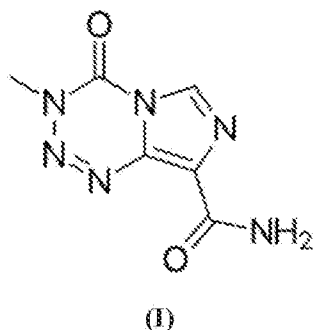
Polymers suitable for use in the monolithic matrix device include naturally occurring polymers, synthetic polymers and synthetically modified natural polymers, as well as polymer derivatives.

- [0280] Any technique known in the art can be used to produce sustained release formulations comprising a presently disclosed AAV vector. See, e.g. U.S. Pat. No. 4,526,938; PCT publication WO 91/05548; PCT publication WO 96/20698; Ning et al., "Intratumoral Radioimmunotherapy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," *Radiotherapy & Oncology*, 39: 179 189, 1996; Song et al., "Antibody Mediated Lung Targeting of Long-Circulating Emulsions," *PDA Journal of Pharmaceutical Science & Technology*, 50: 372 397, 1995; Cleek et al., "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," *Pro. Intl. Symp. Control. Rel. Bioact. Mater.*, 24: 853 854, 1997; and Lam et al., "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," *Proc. Int'l. Symp. Control Rel. Bioact. Mater.*, 24: 759 760, 1997, each of which is incorporated herein by reference in its entirety. In one embodiment, a pump may be used in a controlled release system (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.*, 14: 20, 1987; Buchwald et al., *Surgery*, 88: 507, 1980; and Saudek et al., *N. Engl. J. Med.*, 321: 574, 1989). In certain embodiment, polymeric materials can be used to achieve controlled release of agents comprising dual antigen-binding molecule, or antigen-binding fragments thereof (see e.g., *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, N.Y. (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.*, 23: 61, 1983; see also Levy et al., *Science*, 228: 190, 1985; During et al., *Ann. Neurol.*, 25: 351, 1989; Howard et al., *J. Neurosurg.*, 71: 105, 1989); U.S. Pat. No. 5,679,377; U.S. Pat. No. 5,916,597; U.S. Pat. No. 5,912,015; U.S. Pat. No. 5,989,463; U.S. Pat. No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). In certain embodiment, a controlled release system can be placed in proximity of the therapeutic target (e.g., an affected joint), thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115 138 (1984)). Other controlled release systems are discussed in the review by Langer, *Science*, 249: 1527 1533, 1990.
- 5.8.1 Combination therapy

- [0281] Additional therapies that can be used in a combination with a recombinant AAV described herein or a composition thereof for the treatment of glioma include, but are not limited to, small molecules, synthetic drugs, peptides (including cyclic peptides), polypeptides, proteins, nucleic acids (e.g., DNA and RNA nucleotides including, but not limited to, antisense nucleotide sequences, triple helices, RNAi, and nucleotide

sequences encoding biologically active proteins, polypeptides or peptides), antibodies, synthetic or natural inorganic molecules, mimetic agents, and synthetic or natural organic molecules.

[0282] In a specific embodiment, the additional therapy is a chemotherapeutic agent. In one aspect of the present disclosure, provided is a method of treating a subject having a glioma, comprising administering to the subject a combination therapy, wherein the combination therapy comprises a therapeutically effective amount of a pharmaceutical composition comprising a recombinant AAV encoding NeuroD1 and a therapeutically effective amount of a compound of formula (I)



or solvate, hydrate, tautomer, or a pharmaceutically acceptable salt thereof.

[0283] In some embodiment, the recombinant AAV and the temozolomide compound, or solvate, hydrate, tautomer, or a pharmaceutically acceptable salt thereof, can be administered at the same time (e.g., as part of the same pharmaceutical composition, or in separate pharmaceutical compositions) or at different times, as described herein.

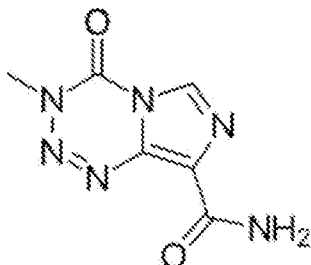
[0284] Pharmaceutically acceptable salt forms include pharmaceutically acceptable acidic/anionic or basic/cationic salts. Pharmaceutically acceptable acidic/anionic salts include acetate, benzenesulfonate, benzoate, bicarbonate, bitartrate, bromide, calcium edetate, camsylate, carbonate, chloride, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, glyceptate, gluconate, glutamate, glycolylarsanilate, hexylresorcinate, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, malonate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, pamoate, pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, hydrogensulfate, tannate, tartrate, teoclate, tosylate, and triethiodide salts. Pharmaceutically acceptable basic/cationic salts include, the sodium, potassium, calcium, magnesium, diethanolamine, N-methyl-D-glucamine, L-lysine, L-arginine, ammonium, ethanolamine, piperazine and triethanolamine salts.

[0285] A pharmaceutically acceptable acid salt is formed by reaction of the free base form of a compound of Formula (I) with a suitable inorganic or organic acid including, but not limited to, hydrobromic, hydrochloric, sulfuric, nitric, phosphoric, succinic, maleic,

formic, acetic, propionic, fumaric, citric, tartaric, lactic, benzoic, salicylic, glutamic, aspartic, p-toluenesulfonic, benzenesulfonic, methanesulfonic, ethanesulfonic, naphthalenesulfonic such as 2-naphthalenesulfonic, or hexanoic acid. A pharmaceutically acceptable acid addition salt of a compound of Formula (I) can comprise or be, for example, a hydrobromide, hydrochloride, sulfate, nitrate, phosphate, succinate, maleate, formate, acetate, propionate, fumarate, citrate, tartrate, lactate, benzoate, salicylate, glutamate, aspartate, p-toluenesulfonate, benzenesulfonate, methanesulfonate, ethanesulfonate, naphthalenesulfonate (e.g., 2-naphthalenesulfonate) or hexanoate salt.

[0286] The free acid or free base forms of the compound of formula (I) may be prepared from the corresponding base addition salt or acid addition salt form, respectively. For example, a compound of the Formula (I) in an acid addition salt form may be converted to the corresponding free base form by treating with a suitable base (e.g., ammonium hydroxide solution, sodium hydroxide, and the like). A compound of Formula (I) in a base addition salt form may be converted to the corresponding free acid by treating with a suitable acid (e.g., hydrochloric acid, etc.).

[0287] In a related aspect, provided herein is also pharmaceutical composition comprising a therapeutically effective amount of a recombinant AAV encoding NeuroD1 and a therapeutically effective amount of a compound of formula (I)



(I),

or solvate, hydrate, tautomer, or a pharmaceutically acceptable salt thereof.

[0288] Additional non-limiting examples of chemotherapeutic agents that may be used in combination with a recombinant AAV described herein or a composition thereof include lomustine (CCNU) and carmustine (BCNU).

[0289] Additional non-limiting examples of chemotherapeutic agents that may be used in combination with a recombinant AAV described herein or a composition thereof also include microtubule disassembly blocker, antimetabolite, topoisomerase inhibitor, and DNA crosslinker or damaging agent. Chemotherapeutic agents that are microtubule disassembly blockers include, but are not limited to, taxenes (e.g., paclitaxel (branded/ marketed as TAXOL®), docetaxel, abraxane, larotaxel, ortataxel, and tesetaxel);

epothilones (e.g., ixabepilone) ; and vinca alkaloids (e.g., vinorelbine, vinblastine, vindesine, and vincristine (branded/marketed as ONCOVIN®)) .

[0290] Chemotherapeutic agents that are antimetabolites include, but are not limited to, folate antimetabolites (e.g., methotrexate, aminopterin, pemetrexed, raltitrexed) ; purine antimetabolites (e.g., cladribine, clofarabine, fludarabine, mercaptopurine, pentostatin, thioguanine) ; pyrimidine antimetabolites (e.g., 5-fluorouracil, capecitabine, gemcitabine (GEMZAR®), cytarabine, decitabine, floxuridine, tegafur) ; and deoxyribonucleotide antimetabolites (e.g., hydroxyurea) .

[0291] In some embodiments, a recombinant AAV described herein or a composition thereof is used in combination with one or more hormonal agents (e.g., aromatase inhibitor, selective estrogen receptor modulator (SERM) , and estrogen receptor antagonist) . Non-limiting examples of hormonal agents that may be used in combination with a recombinant AAV described herein or a composition thereof include aromatase inhibitors, SERMs, and estrogen receptor antagonists. Hormonal agents that are aromatase inhibitors may be steroidal or nonsteroidal. Non-limiting examples of nonsteroidal hormonal agents include letrozole, anastrozole, aminoglutethimide, fadrozole, and vorozole. Non-limiting examples of steroidal hormonal agents include aromasin (exemestane) , formestane, and testolactone. Non-limiting examples of hormonal agents that are SERMs include tamoxifen (branded/marketed as Nolvadex®) , afimoxifene, arzoxifene, bazedoxifene, clomifene, femarelle, lasofoxifene, ormeloxifene, raloxifene, and toremifene. Non-limiting examples of hormonal agents that are estrogen receptor antagonists include fulvestrant. Other hormonal agents include but are not limited to abiraterone and lonaprisan.

[0292] In some embodiments, a recombinant AAV described herein or a composition thereof is used in combination with one or more anti-angiogenic agents (e.g., VEGF antagonist, receptor antagonist, integrin antagonist, vascular targeting agent (VTA) / vascular disrupting agent (VDA)) .

[0293] In some embodiments, a recombinant AAV described herein or a composition thereof is used in combination with conventional surgery.

[0294] In some embodiments, a recombinant AAV described herein or a composition thereof is used in combination with radiation therapy comprising the use of x-rays, gamma rays and other sources of radiation to destroy cancer cells. In specific embodiments, the radiation therapy is administered as external beam radiation or teletherapy, wherein the radiation is directed from a remote source. In other embodiments, the radiation therapy is administered as internal therapy or brachytherapy wherein a radioactive source is placed inside the body close to cancer cells and/or a tumor mass.

[0295] Currently available cancer therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the Physician's Desk Reference (67th ed., 2013) .

EMBODIMENTS

Embodiment 1. A single-stranded self-complementary nucleic acid molecule encoding a NeuroD1

polypeptide, wherein the nucleic acid molecule comprises an expression cassette comprising a coding sequence and one or more regulatory elements operably linked to the coding sequence, wherein the NeuroD1 polypeptide comprises an amino acid sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO: 1.

Embodiment 2. The nucleic acid molecule of embodiment 1, wherein the NeuroD1 polypeptide

comprises an amino acid sequence set forth in SEQ ID NO: 4.

Embodiment 3. The nucleic acid molecule of embodiment 2, wherein the coding sequence comprises

the nucleotide sequence set forth in SEQ ID NO: 5 or a codon-optimized variant thereof.

Embodiment 4. The nucleic acid molecule of any one of embodiments 1 to 3, wherein the one or

more transcription regulatory elements comprise a chimeric intron.

Embodiment 5. The nucleic acid molecule of embodiment 4, wherein the chimeric intron comprises

the sequence set forth in SEQ ID NO: 8.

Embodiment 6. The nucleic acid molecule of any one of embodiments 1 to 5, wherein the one or

more transcription regulatory elements further comprise a CMV promoter comprising the sequence set forth in SEQ ID NO: 7.

Embodiment 7. The nucleic acid molecule of any one of embodiments 1 to 6, wherein the one or

more transcription regulatory elements further comprises a CMV enhancer comprising the sequence set forth in SEQ ID NO: 6.

Embodiment 8. The nucleic acid molecule of any one of embodiments 1 to 7, wherein the one or

more transcription regulatory elements further comprise a polyadenylation (poly-A) signal comprising the sequence set forth in SEQ ID NO: 9.

Embodiment 9. The nucleic acid molecule of any one of embodiment 1 to 8, further comprises a pair

of terminal reverse-complementary fragments each located on one end of the nucleic acid molecule, an anti-sense region that is reverse-complementary to the expression cassette, and a central reverse-complementary fragment that connects the expression cassette and anti-sense region, wherein each of the pair of terminal reverse-complementary fragments and the central reverse-complementary fragment is capable of forming a stem-loop structure.

Embodiment 10. The nucleic acid molecule of embodiment 9, wherein at least one of the pair of terminal reverse-complementary fragments comprises a first inverted terminal repeat (ITR) of a first AAV genome.

Embodiment 11. The nucleic acid molecule of embodiment 10, wherein the first ITR is the 5' ITR of the first AAV genome.

Embodiment 12. The nucleic acid molecule of embodiment 10 or 11, wherein the first AAV genome is the AAV2 genome.

Embodiment 13. The nucleic acid molecule of embodiment 9, wherein the pair of terminal reverse-complementary fragments comprises the sequence set forth in SEQ ID NO: 13 and SEQ ID NO: 36, respectively.

Embodiment 14. The nucleic acid molecule of any one of embodiments 9 to 13, wherein the central reverse-complementary fragment comprises a second ITR of a second AAV genome.

Embodiment 15. The nucleic acid molecule of embodiment 14, wherein the central reverse-complementary fragment comprises the 3' ITR of the second AAV genome, wherein the 3' ITR is mutated to disrupt a terminal resolution site (trs).

Embodiment 16. The nucleic acid molecule of embodiment 14 or 15, wherein the second AAV genomes is the AAV2 genome.

Embodiment 17. The nucleic acid molecule of any one of embodiments 9 to 13, wherein the central reverse-complementary fragment comprises the sequence set forth in SEQ ID NO: 14.

Embodiment 18. The nucleic acid molecule of embodiment 1, comprising the sequence set forth in SEQ ID NO: 16.

Embodiment 19. The nucleic acid molecule of any one of embodiments 1 to 18, wherein the nucleic

acid molecule is DNA.

Embodiment 20. A single-stranded self-complementary DNA molecule consists of the sequence set

forth in SEQ ID NO: 16.

Embodiment 21. A recombinant adeno-associated virus (rAAV) comprising a self-complementary

genome, wherein the self-complementary genome comprises the DNA molecule of any one of embodiments 1 to 20.

Embodiment 22. The recombinant AAV of embodiment 21, wherein the recombinant AAV comprises

a AAV serotype 6 (AAV6) capsid.

Embodiment 23. The recombinant AAV of embodiment 21 or 22, wherein the AAV6 capsid comprises

capsid proteins selected from the group of AAV6 VP1 polypeptides, AAV6 VP2 polypeptides and AAV6 VP3 polypeptides.

Embodiment 24. The recombinant AAV of embodiment 22, wherein the AAV6 capsid comprises

AAV6 VP1 comprising the amino acid sequence set forth in SEQ ID NO: 25.

Embodiment 25. The recombinant AAV of embodiment 22 or 24, wherein the AAV6 capsid further

comprises AAV6 VP2 comprising the amino acid sequence set forth in SEQ ID NO: 26.

Embodiment 26. The recombinant AAV of any one of embodiments 22, 24, or 25, wherein the AAV6

capsid further comprises AAV6 VP3 comprising the amino acid sequence set forth in SEQ ID NO: 27.

Embodiment 27. A pharmaceutical composition comprising the recombinant AAV of any one of

embodiments 21 to 26, wherein the pharmaceutical composition further comprises:

(a) potassium chloride,

(b) potassium phosphate monobasic,

(c) sodium chloride,

(d) sodium phosphate dibasic anhydrous, and

(e) poloxamer 188, polysorbate 20, or polysorbate 80.

Embodiment 28. A pharmaceutical composition consists of:

(a) a recombinant adeno-associated virus (AAV) ,

(b) sodium chloride at a concentration of about 180 mM;

(c) sodium phosphate at a concentration of about 10 mM; and

(d) poloxamer 188 at a concentration of about 0.001%weight/volume (0.01 g/L) ; and wherein the pH of the pharmaceutical composition is about 7.3.

Embodiment 29. A pharmaceutical composition consists of:

(a) a recombinant adeno-associated virus (AAV) ,
 (b) sodium chloride at a concentration of about 200 mM;
 (c) magnesium chloride at a concentration of about 1 mM;
 (d) Tris hydrochloride at a concentration of about 20 mM, and
 (e) poloxamer 188 at a concentration of about 0.005%weight/volume (0.05 g/L) ; and wherein the pH of the pharmaceutical composition is about 8.0.

Embodiment 30. A pharmaceutical composition consists of:

(a) a recombinant adeno-associated virus (AAV) ,
 (b) sodium chloride at a concentration of about 150 mM;
 (c) calcium chloride at a concentration of about 1.4 mM;
 (d) magnesium chloride at a concentration of about 0.8 mM,
 (e) sodium phosphate at a concentration of about 1 mM, and
 (f) poloxamer 188 at a concentration of about 0.001%weight/volume (0.01 g/L) ; and wherein the pH of the pharmaceutical composition is about 7.4.

Embodiment 31. The pharmaceutical composition of any one of embodiments 28 to 30, wherein

recombinant AAV is the recombinant AAV of any one of embodiments 21 to 26.

Embodiment 32. The pharmaceutical composition of any one of embodiments 27 to 31, wherein the recombinant AAV is AAV6.

Embodiment 33. The pharmaceutical composition of any one of embodiments 27 to 32, wherein a

vector genome concentration of the recombinant AAV in the pharmaceutical composition is in the range of about 5×10^{11} to about 1×10^{14} viral genomes per mL (vg/mL) ; optionally, wherein the vector genome concentration is about 1×10^{12} vg/mL, about 1.5×10^{12} vg/mL, about 2×10^{12} vg/mL, about 2.5×10^{12} vg/mL, about 3×10^{12} vg/mL, about 3.5×10^{12} vg/mL, about 4×10^{12} vg/mL, about 4.5×10^{12} vg/mL, about 5×10^{12} vg/mL, about 5.5×10^{12} vg/mL, about 6×10^{12} vg/mL, about 6.5×10^{12} vg/mL, about 6.6×10^{12} vg/mL, about 7×10^{12} vg/mL, about 7.5×10^{12} vg/mL, about 8×10^{12} vg/mL, about 8.5×10^{12} vg/mL, about 9×10^{12} vg/mL, about 9.5×10^{12} vg/mL, about 1×10^{13} vg/mL, about 1.5×10^{13} vg/mL, about 2×10^{13} vg/mL, about 2.5×10^{13} vg/mL,

about 3×10^{13} vg/mL, about 3.5×10^{13} vg/mL, about 4×10^{13} vg/mL, about 4.5×10^{13} vg/mL, about 5×10^{13} vg/mL, about 5.5×10^{13} vg/mL, about 6×10^{13} vg/mL, about 6.5×10^{13} vg/mL, about 7×10^{13} vg/mL, about 7.5×10^{13} vg/mL, about 8×10^{13} vg/mL, about 8.5×10^{13} vg/mL, about 9×10^{13} vg/mL, about 9.5×10^{13} vg/mL, or about 1×10^{14} vg/mL. Embodiment 34. A method for treating glioma, comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising a recombinant AAV, wherein the recombinant AAV comprises a self-complementary genome comprising a transgene encoding a NeuroD1 polypeptide.

Embodiment 35. The method of embodiment 34, wherein the self-complementary genome is a single-stranded DNA molecule comprising a pair of terminal reverse-complementary fragments each located on one end of the DNA molecule, an expression cassette comprising a coding sequence of the transgene, an anti-sense region that is reverse complementary to the expression cassette, and a central reverse-complementary fragment that connects the expression cassette and anti-sense region; and wherein each of the pair of terminal reverse-complementary fragments and the central reverse-complementary fragment is capable of forming a stem-loop structure.

Embodiment 36. The method of embodiment 34 or 35, wherein the NeuroD1 polypeptide comprises an amino acid sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO: 1.

Embodiment 37. The method of embodiment 34 or 35, wherein the coding sequence of the transgene comprises the nucleotide sequence set forth in SEQ ID NO: 5 or a codon-optimized version thereof.

Embodiment 38. The method of any one of embodiments 35 to 37, wherein the expression cassette further comprises one or more transcription regulatory elements operably linked to the coding sequence of the transgene.

Embodiment 39. The method of embodiment 38, wherein the one or more transcription regulatory elements comprise a chimeric intron.

Embodiment 40. The method of embodiment 39, wherein the chimeric intron comprises the sequence of SEQ ID NO: 8.

Embodiment 41. The method of any one of embodiments 38 to 40, wherein the one or more

transcription regulatory elements further comprise a CMV promoter comprising the sequence of SEQ ID NO: 7; optionally the one or more transcription regulatory element further comprises a CMV enhancer comprising the sequence of SEQ ID NO: 6.

Embodiment 42. The method of any one of embodiments 38 to 41, wherein the one or more

transcription regulatory elements further comprise a polyadenylation (poly-A) signal comprising the sequence of SEQ ID NO: 9.

Embodiment 43. The method of any one of embodiments 35 to 42, wherein at least one of the pair of

terminal reverse-complementary fragments comprises a first inverted terminal repeat (ITR) of a first AAV genome; optionally wherein the first ITR is the 5' ITR of the first AAV genome.

Embodiment 44. The method of embodiment 43, wherein the first AAV genome is the AAV2 genome.

Embodiment 45. The method of any one of embodiments 35 to 42, wherein the pair of terminal

reverse-complementary fragments are reverse complementary to one another.

Embodiment 46. The method of any one of embodiments 35 to 42, wherein the pair of terminal

reverse-complementary fragments comprises the sequence set forth in SEQ ID NO: 13 and SEQ ID NO: 36, respectively.

Embodiment 47. The method of any one of embodiments 35 to 46, wherein the central reverse-

complementary fragment comprises a second ITR of a second AAV genome.

Embodiment 48. The method of embodiment 47, wherein the central reverse-complementary fragment

comprises the 3' ITR of the second AAV genome, wherein the 3' ITR is mutated to disrupt a terminal resolution site (trs) .

Embodiment 49. The method of embodiment 47 or 48, wherein the second AAV genomes is the AAV2 genome.

Embodiment 50. The method of any one of embodiments 35 to 46, wherein the central reverse-

complementary fragment comprises the sequence set forth in SEQ ID NO: 14.

Embodiment 51. The method of embodiment 34, wherein the self-complementary genome of the recombinant AAV comprises the sequence set forth in SEQ ID NO: 16.

Embodiment 52. The method of any one of embodiments 34 to 51, wherein the recombinant AAV comprises an AAV serotype 6 (AAV6) capsid.

Embodiment 53. The method of embodiment 52, wherein the AAV6 capsid comprises capsid proteins selected from the group of AAV6 VP1 polypeptides, AAV6 VP2 polypeptides and AAV6 VP3 polypeptides.

Embodiment 54. The method of embodiment 52, wherein the AAV6 capsid comprises AAV6 VP1 comprising the amino acid sequence set forth in SEQ ID NO: 25.

Embodiment 55. The method of embodiment 52 or 54, wherein the AAV6 capsid further comprises AAV6 VP2 comprising the amino acid sequence set forth in SEQ ID NO: 26.

Embodiment 56. The method of any one of embodiments 52, 54, and 55, wherein the AAV6 capsid further comprises AAV6 VP3 comprising the amino acid sequence set forth in SEQ ID NO: 27.

Embodiment 57. The method of any one of embodiments 34 to 56, wherein the pharmaceutical composition is administered to the subject intratumorally.

Embodiment 58. The method of any one of embodiments 34 to 56, wherein the subject has a surgical cavity created by removing a glioma surgically, and wherein the pharmaceutical composition is administered to the surgical cavity.

Embodiment 59. The method of any one of embodiments 34 to 58, wherein the pharmaceutical

composition comprises from about 5×10^{11} to about 1×10^{14} viral genomes (vg) of the recombinant AAV.

Embodiment 60. The method of any one of embodiments 34 to 58, wherein the pharmaceutical composition comprises about 2×10^{12} vg of the recombinant AAV.

Embodiment 61. The method of any one of embodiments 34 to 58, wherein the pharmaceutical

composition comprises about 6.6×10^{12} vg of the recombinant AAV.

Embodiment 62. The method of any one of embodiments 34 to 58, wherein the pharmaceutical

composition comprises about 2×10^{13} vg of the recombinant AAV.

Embodiment 63. The method of any one of embodiments 34 to 58, wherein the pharmaceutical

composition comprises about 4×10^{12} vg of the recombinant AAV.

Embodiment 64. The method of any one of embodiments 34 to 58, wherein the pharmaceutical

composition comprises about 1.32×10^{13} vg of the recombinant AAV.

Embodiment 65. The method of any one of embodiments 34 to 58, wherein the pharmaceutical

composition comprises about 4×10^{13} vg of the recombinant AAV.

Embodiment 66. The method of any one of embodiments 34 to 56, wherein the subject is administered

intratumorally the pharmaceutical composition comprising about 2×10^{12} vg of the recombinant AAV once every 15 days for once, twice or at least three times; optionally wherein for each administration the subject is administered about 1 mL of the pharmaceutical composition comprising about 2×10^{12} vg/mL of the recombinant AAV intratumorally; optionally wherein the pharmaceutical composition is administered through a Ommaya reservoir.

Embodiment 67. The method of any one of embodiments 34 to 56, wherein the subject is administered

intratumorally the pharmaceutical composition comprising about 6.6×10^{12} vg of the recombinant AAV once every 15 days for once, twice or at least three times; optionally wherein for each administration the subject is administered about 1 mL of the pharmaceutical composition comprising about 6.6×10^{12} vg/mL of the recombinant AAV intratumorally; optionally wherein the pharmaceutical composition is administered through a Ommaya reservoir.

Embodiment 68. The method of any one of embodiments 34 to 56, wherein the subject is administered

intratumorally the pharmaceutical composition comprising about 2×10^{13} vg of the recombinant AAV once every 15 days for once, twice or at least three times; optionally wherein for each administration the subject is administered about 1 mL of the pharmaceutical composition comprising about 2×10^{13} vg/mL of the recombinant AAV intratumorally; optionally wherein the pharmaceutical composition is administered through a Ommaya reservoir.

Embodiment 69. The method of any one of embodiments 34 to 56, wherein the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 4×10^{12} vg of the recombinant AAV to the surgical cavity once every 30 days for once, twice or at least three times; optionally wherein for each administration the subject is administered about 2 mL of the pharmaceutical composition comprising about 2×10^{12} vg/mL of the recombinant AAV to the surgical cavity; optionally wherein the pharmaceutical composition is administered through a Ommaya reservoir.

Embodiment 70. The method of any one of embodiments 34 to 56, wherein the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 1.32×10^{13} vg of the recombinant AAV to the surgical cavity once every 30 days for once, twice or at least three times; optionally wherein for each administration the subject is administered about 2 mL of the pharmaceutical composition comprising about 6.6×10^{12} vg/mL of the recombinant AAV to the surgical cavity; optionally wherein the pharmaceutical composition is administered through a Ommaya reservoir.

Embodiment 71. The method of any one of embodiments 34 to 56, wherein the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 4×10^{13} vg of the recombinant AAV to the surgical cavity once every 30 days for once, twice or at least three times; optionally wherein for each administration the subject is administered about 2 mL of the pharmaceutical composition comprising about 2×10^{13} vg/mL of the recombinant AAV to the surgical cavity; optionally wherein the pharmaceutical composition is administered through a Ommaya reservoir.

Embodiment 72. The method of any one of embodiments 34 to 71, wherein the pharmaceutical composition further comprises:

- (a) potassium chloride,
- (b) potassium phosphate monobasic,
- (c) sodium chloride,
- (d) sodium phosphate dibasic anhydrous, and
- (e) poloxamer 188, polysorbate 20, or polysorbate 80.

Embodiment 73. The method of any one of embodiments 34 to 71, wherein the pharmaceutical

composition further comprises:

- (a) sodium chloride at a concentration of about 180 mM;
 - (b) sodium phosphate at a concentration of about 10 mM; and
 - (c) poloxamer 188 at a concentration of about 0.001%weight/volume (0.01 g/L) ; and
- wherein the pH

of the pharmaceutical composition is about 7.3.

Embodiment 74. The method of any one of embodiments 34 to 71, wherein the pharmaceutical

composition further comprises:

- (a) sodium chloride at a concentration of about 200 mM;
 - (b) magnesium chloride at a concentration of about 1 mM;
 - (c) Tris hydrochloride at a concentration of about 20 mM, and
 - (d) poloxamer 188 at a concentration of about 0.005%weight/volume (0.05 g/L) ; and
- wherein the pH

of the pharmaceutical composition is about 8.0.

Embodiment 75. The method of any one of embodiments 34 to 71, wherein the pharmaceutical

composition further comprises:

- (a) sodium chloride at a concentration of about 150 mM;
 - (b) calcium chloride at a concentration of about 1.4 mM;
 - (c) magnesium chloride at a concentration of about 0.8 mM,
 - (d) sodium phosphate at a concentration of about 1 mM, and
 - (e) poloxamer 188 at a concentration of about 0.001%weight/volume (0.01 g/L) ; and
- wherein the pH

of the pharmaceutical composition is about 7.4.

Embodiment 76. The method of any one of embodiments 34 to 75, wherein the glioma is selected from

adult-type diffuse gliomas; astrocytoma, IDH-mutant; oligodendroglioma, IDH-mutant, and 1p/19q-codeleted; glioblastoma, IDH-wildtype; pediatric-type diffuse low-grade gliomas; diffuse astrocytoma, MYB-or MYBL1-altered; angiocentric glioma; polymorphous low-grade neuroepithelial tumor of the young; diffuse low-grade glioma, MAPK pathway-altered; pediatric-type diffuse high-grade gliomas; diffuse midline glioma, H3 K27-altered; diffuse hemispheric glioma, H3 G34-mutant; diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype; infant-type hemispheric glioma; circumscribed astrocytic gliomas; pilocytic astrocytoma; high-grade astrocytoma with piloid features; pleomorphic xanthoastrocytoma; subependymal giant cell astrocytoma; chordoid glioma; astroblastoma, MN1-altered; ganglioglioma; and esmoplastic infantile ganglioglioma.

Embodiment 77. The method of any one of embodiments 34 to 75, wherein the glioma is selected from

oligodendroglioma, IDH mutant, and 1p/19q-codeleted (WHO grade 3) ; astrocytoma, IDH mutant (WHO grade 3, 4) , and glioblastoma, IDH wildtype (WHO grade 4) .

Embodiment 78. The method of any one of embodiments 34 to 75, wherein the glioma is selected from

diffuse midline glioma, H3 K27-altered; diffuse hemispheric glioma, H3 G34-mutant; diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype; and infant-type hemispheric glioma. Embodiment 79. The method of any one of embodiments 34 to 78, wherein the subject is treatment naïve for glioma.

Embodiment 80. The method of any one of embodiments 34 to 78, wherein the subject has received a

previous therapy for treating glioma, and the glioma is refractory to or relapsed from the previous therapy.

Embodiment 81. The method of any one of embodiments 34 to 80, wherein the previous therapy is

radiation therapy or chemotherapy.

Embodiment 82. The method of any one of embodiments 34 to 81, wherein the subject is a human.

Embodiment 83. The method of any one of embodiments 34 to 82, wherein upon administering the

pharmaceutical composition, the NeuroD1 polypeptide is expressed by a population of glioma cells.

Embodiment 84. The method of embodiment 83, wherein proliferative activity in the population of

glioma cells reduces; optionally wherein at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95% of the glioma cells in the population stop proliferation; optionally wherein proliferative activity in the population of glioma cells reduces in less than 14 days after the administering of the pharmaceutical composition.

Embodiment 85. The method of embodiment 83, wherein the population of glioma cells exhibit one or

more neuronal phenotypes; optionally wherein the neuronal phenotype comprises expressing one or more neuronal markers selected from DCX, TUJ1, NeuN, and MAP2; optionally the population of glioma cells exhibit the one or more neuronal phenotype in less than 14 days after the administering of the pharmaceutical composition.

Embodiment 86. The method of embodiment 83, wherein the population of glioma cells stop expressing one or more glial marker; optionally the one or more glial marker is selected from GFAP, Aldh1l1, S100 β and Sox9; optionally the population of glioma cells stop expressing the one or more glial marker in less than 14 days after the administering of the pharmaceutical composition.

Embodiment 87. The method of embodiment 83, wherein the population of glioma cells trans-differentiate into neurons; optionally wherein the neurons are selected from glutamatergic neurons, GABAergic neurons, dopaminergic neurons; motor neurons, glycinergic neurons, serotonergic neurons, norepinephrinergic neurons, and sensory neurons; optionally wherein at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95% of the glioma cells in the population transdifferentiate into neurons.

Embodiment 88. The method of embodiment 83, wherein the population of glioma cells form one or more gliomas, and wherein the size of the glioma is reduced; optionally wherein the size of the glioma is reduced for at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95%. Embodiment 89. A gene-of-interest (GOI) plasmid comprising an expression cassette comprising a transgene of interest and a pair of AAV ITR sequences flanking the expression cassette, wherein the AAV ITR located to the 3' end of the expression cassette does not have a functional terminal resolution site (trs), and wherein the transgene encodes a NeuroD1 polypeptide, wherein the NeuroD1 polypeptide comprises an amino acid sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO: 1.

Embodiment 90. A host cell comprising the GOI plasmid of embodiment 89.

Embodiment 91. A method of producing a recombinant AAV comprising:

(a) culturing a host cell containing:

(i) an artificial genome comprising a cis expression cassette, wherein the cis expression

cassette comprises a coding sequence encoding a NeuroD1 polypeptide, wherein the NeuroD1 polypeptide comprises an amino acid sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO: 1;

(ii) a trans expression cassette lacking AAV ITRs, wherein the trans expression cassette

encodes an AAV Rep and Capsid proteins operably linked to expression control element that drive expression of the AAV Rep and capsid proteins in the host cell in culture, and supply the Rep and Capsid proteins in trans;

(iii) sufficient adenovirus helper functions to permit replication and packaging of the artificial genome by the AAV capsid proteins; and

(b) recovering the recombinant AAV encapsidating the artificial genome from the cell culture.

Embodiment 92. The method of embodiment 91, wherein the artificial genome is synthesized by the host cell using a GOI plasmid sequence as a replication template, wherein the GOI plasmid comprises the cis expression cassette flanked by a pair of AAV ITR sequences, wherein the AAV ITR located 3' to the cis expression cassette does not have a functional terminal resolution site (trs) .

Embodiment 93. A host cell comprising an artificial genome comprising the single-stranded self-

complementary nucleic acid molecule of any one of embodiments 1 to 20.

6. EXAMPLES

[0296] The examples in this section (i.e., Section 6) are offered by way of illustration, and not by way of limitation.

6.1 Example 1: Method and Materials

6.1.1 Construction of recombinant AAVs (rAAVs) encoding NeuroD1.

[0297] The AAV genome is packaged as a linear ssDNA molecule with palindromic inverted terminal repeat (ITR) sequences forming dsDNA hairpin structures at each end. The generation of normal monomeric (single-stranded) AAV genomes relies on the efficient resolution of the two ITRs in turn, with each round of DNA synthesis. This reaction is mediated by the ssDNA endonuclease activity of the two larger isoforms of the AAV Rep protein. Nicking the ITR at the terminal resolution site is followed by DNA elongation from the nick by host DNA polymerase. Dimeric genomes are formed when Rep fails to nick the terminal resolution site before it is reached by the replication complex initiated at the other end.

[0298] To produce ssAAV particles, a plasmid carrying the transgene of interest (GOI plasmid) carried the transgene expression elements (including the coding region and regulatory control elements) flanked by wild-type AAV ITR sequences. The GOI plasmid, upon co-transfection of packaging cells (e.g., 293 cells) with one or more plasmid (s) encoding the AAV capsid proteins, as well as functional nucleic acids and proteins of the viral life cycle machinery, was processed into a linear ssDNA molecule with ITR sequences forming dsDNA hairpin structures at each end. The linear ssDNA molecule was assembled with capsid proteins synthesized in the packaging cells into

a virion, where the single-stranded DNA genome was packaged inside the protein capsid.

[0299] The yield of dimeric genomes in a scAAV preparation can be increased dramatically by inhibiting resolution at one terminal repeat. This can be accomplished by deleting the terminal resolution site sequence from one ITR, such that the Rep protein cannot generate the essential ssDNA nick. The replication complex initiated at the other ITR then copies through the hairpin and back toward the initiating end. Replication proceeds to the end of the template molecule, leaving a dsDNA inverted repeat with a wild-type ITR at each end and the mutated ITR in the middle. This dimeric inverted repeat can then undergo normal rounds of replication from the two wild-type ITR ends. Each displaced daughter strand comprises a ssDNA inverted repeat with a complete ITR at each end and a mutated ITR in the middle. Packaging into the AAV capsid ensues from the 3' end of the displaced strand.

[0300] In this study, as shown in FIG. 1, to produce scAAV particles, the GOI plasmid carried, in the continuous order, (a) a full-length AAV left (5') ITR sequence, (b) a CMV promoter including CMV enhancer and CMV core promoter sequences, (c) a chimeric intron sequence, (d) a NeuroD1 coding sequence, (e) a polyadenylation signal, (f) a AAV right (3') ITR sequence with the terminal resolution site (trs) deleted, and (g) plasmid backbone sequence.

[0301] Table 6.1.1 shows the sequences of functional fragments of the GOI plasmid used to produce scAAV encoding NeuroD1 (scAAV-NeuroD1) , and the full-length sequence of the GOI plasmid used in the study.

Table 6.1.1

GOI Plasmid for producing scAAV genome		
AAV2 Left (5') ITR (SEQ ID NO:13)	TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGCAC CAAAGGTCGCCCAGCGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCG AGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCT	
CMV Enhancer (SEQ ID NO:6)	GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCAATT AGTTCATAGCCCATATATGGAGTTCGCGTTACATAAATTACGGTAAAT GGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTTGACGTCAATA ATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC AATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAG TGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATG GCCCCCTGGCATTAATGCCAGTACATGACCTTATGGGACTTTCTTACT TGGCAGTACATCTACGTATTAGTCATCGCTATTACCATG	
CMV Promoter (SEQ ID NO:7)	GTGATGCGGTTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACT CACGGGGATTTCCAAGTCTCCACCCCAATTGACGTCAATGGGAGTTTGT TTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGC CCCATTTGACGCAAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATAT AAGCAGAGCT	
Chimeric Intron (SEQ ID NO:8)	GTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTG GGCTTGTGAGACAGAGAAGACTCTTGCCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCTTTCTCTCCACAG	
Transcriptional factor coding Region	Human NeuroD1 coding sequence (SEQ ID NO:5)	ATGGTGACCAAATCGTACAGCGAGAGTGGGCTGATG GGCGAGCCTCAGCCCCAAGGTCCTCCAAGCTGGACAG ACGAGTGTCTCAGTTCTCAGGACGAGGAGCACGAGGC AGACAAGAAGGAGGACGACCTCGAAACCATGAACGC AGAGGAGGACTCACTGAGGAACGGGGGAGAGGAGGA GGACGAAGATGAGGACCTGGAAGAGGAGGAAGAAG AGGAAGAGGAGGATGACGATCAAAAGUCCCAAGAGAC GCGGCCCAAAAAGAAGAAGATGACTAAGGCTCGCC TGGAGCGTTTFAAATTTGAGACGCATGAAGGCTAACGC CCGGGAGCGGAACCGCATGCACGGACTGAACGCGGC GCTAGACAACCTGCGCAAGGTGGTGCCTTGCTATTCT AAGACGCAGAAGCTGTCCAAAATCGAGACTCTGCGCT TGGCCAAGAACTACATCTGGGCTCTGTGCGGAGATCCT GCGCTCAGGCAAAAGCCCAGACCTGGTCTCCTTCGTT CAGACGCTTTGCAAGGGCTTATCCCAACCCACCACCA ACCTGGTTGCGGGCTGCCTGCAACTCAATCCTCGGAC TTTTCTGCCTGAGCAGAACCAGGACATGCCCCCCCAC CTGCCGACGGCCAGCGCTTCCCTTCCCCTGTACACCCCTA CTCCTACCAGTCGCCTGGGCTGCCAGTCCGCTTAC

		GGTACCATGGACAGCTCCCATGTCTTCCACGTTAAGC CTCCGCCGCACGCCCTACAGCGCAGCGCTGGAGCCCTT CTTTGAAAGCCCTCTGACTGATTGCACCAGCCCTTCCT TTGATGGACCCCTCAGCCCGCCGCTCAGCATCAATGG CAACTTCTCTTTCAAACACGAACCGTCCGCCGAGTTT GAGAAAAATTATGCCTTTACCATGCACTATCCTGCAG CGACACTGGCAGGGGGCCCAAGCCACGGATCAATCTT CTCAGGCACCGCTGCCCCCTCGCTGCGAGATCCCCATA GACAATATTATGTCCTTCGATAGCCATTACATCATG AGCGAGTCATGAGTGCCCAGCTCAATGCCATATTTCA TGATTGA
	Encoded human NeuroD1 amino acid sequence (SEQ ID NO:4)	MVTKSYSESGLMGEPQPQGPSPWTDECLSSQDEEHEAD KKEDDLETMNAEEDSLRNGGEEDEDEDELEEEEEEEEE DDDQKPKRRGPKKKKMTKARLERFKLRMRKANARERN RMHGLNAALDNLRKVVPCYSKTQKLSKIETLRLAKNYI WALSEILRSGKSPDLVSFVQTLCKGLSQPTTNLVAGCLQ LNPRTFLPEQNQDMPPHLPTASASFPVHPYSYQSPGLPSP PYGTMDSSHVFHVKPPPHAYSAALEPFFESPLTDCITSPSF DGPLSPPLSINGNFSFKHEPSAEFEKNYAFTMHYPATL AGAQSHGSIFSGTAAPRCEIPIDNIMSFDSHSHHERVMSA QLNAIFHD
Polyadenylation signal (SEQ ID NO:9)	CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTCCCCCTCCCCCGTGCCT TCCTTGACCCTGGAAGGTGCCACTCCCACTGTCTTTCTAATAAAAATG AGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGG TGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGAGAATAGCA GGCATGCTGGGGA	
Mutated AAV2 right ITR with trs deleted (SEQ ID NO:14)	CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAA GGTCGCCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCG AGCGCGCAGAGAGGGAGTGG	
Plasmid backbone (SEQ ID NO:17)	ATCCCAATGGCGCGCCGAGCTTGGCTCGAGCATGGTCATAGCTGTTTCC TGTTGTGAAATGTGTTATCCGCTCACAATTCACACACAACATACGAGCCGGA AGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACA TTAATTGCGTTGCGCTCACTGCCCGCTTTCAGTCGGGAAACCTGTCTGT GCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGC GTATTGGGCGCTGTTCCGCTTCTCCTCGCTCACTGACTCGCTGCGCTCGGT CGTTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACG GTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAA AAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTTGTCTGGCG TTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAATAATCGACGCT CAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCG TTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCGGACCCCTGCCGCT TACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCT CATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCA AGCTGGGCTGTGTGCACGAACCCCGTTTCAGCCCCGACCGCTGCGCCTT ATCCGGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCG CCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTA	

	GGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACT AGAAAGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCG GAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCACCGCTGGTA GCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAG GATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTG GAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAAG GATCTTCACCTAGATCCTTTTAAATTAATAAATGAAGTTTTAAATCAATC TAAAGTATATATGAGTAAACTTGGTCTGACAGTTAGAAAAACTCATCG AGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCAT ATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGC AGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCG TCCAACATCAATACAACCTATTAATTTCCCTCGTCAAAAAATAAGGTTA TCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGCC AAAAGTTTATGCATTTCTTTCCAGACTTGTTTCAACAGGCCAGCCATTAC GCTCGTCATCAAAATCACTCGCATCAACCAAAACCGTTATTCATTCTGTA TTGCGCCTGAGCGAAACGAAATACGCGATCGCTGTTAAAAGGACAATT ACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCAT CAACAATAATTTACCTGAATCAGGATATTCTTCTAATACCTGGAATGC TGTTTTCCAGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGT ACGGATAAAATGCTTGATGGTCCGAAGAGGCATAAATTCGGTCAGCCA GTTTAGTCTGACCATCTCATCTGTAAACATCATTTGGCAACGCTACCTTTG CCATGTTTCAGAAACAACCTCTGGCGCATCGGGCTTCCCATACAATCGAT AGAATTGTCGCACCTGATTTGCCCGACATTTATCGCGAGCCCAATTTATACCC ATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTAGAGCAAGA CGTTTCCCGTTGAATATGGCTCATACTCTTCTTTTCAATATTATTGAA GCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTAT TTAGAAAAATAAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGT GCCACCTGACGTCTAAGAAACCAATTATTATCATGACATTAACCTATAAA AATAGGCGTATCACGAGGCCCTTTTGTCTCGCGCGTTTCGGTGATGACG GTGAAAACCTCTGACACATGACAGCTCCCGGAGACTGTCAAGCTTGICT GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGCGCGCTCAGCGG GTGTGGCGGGTGTGCGGGCTGGCTTAACTATGCGGCATCAGAGCAGA TTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCG TAAGGAGAAAATACCGCATCAGGCGCCATTTCGCCATTACGGCTGCGCA ACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCT GGCGAAAGGGGGATGTGCTGCAAGCCGATTAAGTTGGGTAAACGCCAG GGTTTCCAGTCACGACGTGTGTAACGACGGCCAGTGAATTGACGC GTATTGGGAT
Full-length NeuroD1 GOI Sequence (SEQ ID NO:18)	GGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCA AAGGTCGCCCCGACGCCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAG CGAGCGCGCAGAGAGGGAGTGGCCAACCTCCATCACTAGGGGTTCTGA CATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTTCATTAG TTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGG CCCCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAAT GACGTAATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAA TGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGT ATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGC CCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTTACTTG GCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTT TGGCAGTACATCAATGGGCGTGGATAGCGGTTTGAATCACGGGGATTT

CCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTGTTTGGCACCAAA
ATCAACGGGACTTTICCAAAATGTGTAACAACITCCGCCCCATTGACGC
AAATGGGCGGTAGGCGTGACGGTGGGAGGTCTATATAAGCAGAGCTG
TAAGTATCAAGGTTACAAGACAGGTTTAAAGGAGACCAATAGAAACTGG
GCTTGTGAGACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGG
TCTTACTGACATCCACTTTGCCTTCTCTCCACAGATGGTGACCAAAATC
GTACAGCGAGAGTGGGCTGATGGGCGAGCCTCAGCCCCAAGGTCTCC
AAGCTGGACAGACGAGTGTCTCAGTTCTCAGGACGAGGAGCACGAGGC
AGACAAGAAGGAGGACGACCTCGAAACCATGAACGCAGAGGAGGACT
CACTGAGGAACGGGGGAGAGGAGGAGGACGAAGATGAGGACCTGGAA
GAGGAGGAAGAAGAGGAAGAGGAGGATGACGATCAAAAGCCCAAGA
GACGCGGCCCCAAAAAGAAGAAGATGACTAAGGCTCGCCTGGAGCGTT
TTAAATTGAGACGCATGAAGGCTAACGCCCGGGAGCGGAACCGCATGC
ACGGACTGAACGCGGCGCTAGACAAACCTGCGCAAGGTGGTGCCTTGCT
ATTCTAAGACGCAGAAGCTGTCCAAAATCGAGACTCTGCGCTTGGCCA
AGAACTACATCTGGGCTCTGTGCGAGATCCTGCGCTCAGGCAAAAGCC
CAGACCTGGTCTCCTTCGTTTCAGACGCTTTGCAAGGGCTTATCCCAACC
CACCACCAACCTGGTTGCGGGCTGCCTGCAACTCAATCCTCGGACTTTT
CTGCCTGAGCAGAACCAGGACATGCCCCCACCCTGCCGACGGCCAGC
GCTTCCTTCCCTGTACACCCCTACTCCTACCAGTCGCTTGGGCTGCCCA
GTCCGCTTACGGTACCATGGACAGCTCCCATGTCTTCCACGTTAAGCC
TCCGCCGCACGCCTACAGCGCAGCGCTGGAGCCCTTCTTTGAAAGCCCT
CTGACTGATTGACCCAGCCCTTCTTTGATGGACCCCTCAGCCCGCCGC
TCAGCATCAATGGCAACTTCTCTTCAAACACGAACCGTCCGCCGAGTT
TGAGAAAAATTATGCCTTTACCATGCACTATCCTGCAGCGACACTGGCA
GGGGCCCCAAAGCCACGGATCAATCTTCTCAGGCACCGCTGCCCTCGC
TGCGAGATCCCCATAGACAATATTATGTCTTCGATAGCCATTACATC
ATGAGCGAGTCATGAGTGGCCAGCTCAATGCCATATTTTCATGATTGACT
GTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTC
CTTGACCCTGGAAAGGTGGCACTCCCACTGTCTTCTTAATAAAATGAG
GAAATTGCATCGCATTTGTCTGAGTAGGTGTCTATTCTATTCTGGGGGTG
GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGAGAATAGCAGG
CATGCTGGGGACCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCC
GGGCGACCAAAGGTGCCCCGACGCCCCGGGCTTTGCCCCGGCGGCCCTCA
GTGAGCGAGCGAGCGCGCAGAGAGGGAATCCCAATGGCGCGCCGAGC
TTGGCTCGAGCATGGTCATAGCTGTTTCTGTGTGAAATTGTTATCCGC
TCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCT
GGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACT
GCCCCGCTTTCCAGTGGGAAACCTGTCTGTGCCAGCTGCAITTAATGAATC
GGCCAACGCGCGGGGAGAGGCGGTTTGCCTATTGGGCGCTGTTCCGCT
TCCTCGCTCACTGACTCGCTGCGCTCGGTCTGCGCTGCGGCGAGCGG
TATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGG
ATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGG
AACCGTAAAAAGGCCGCGTTTGTGGCGTTTTCCTAAGGCTCCGCCCCC
CTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACC
CGACAGGACTATAAAGATAACAGGCGTTTCCCCCTGGAAGCTCCCTCG
TGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTT
CTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATC
TCAGTTGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACC
CCCCGTTACGCCCCGACGCTGCGCTTATCCGGTAACATCGTCTTGAG
TCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGT

	AACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTG AAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATC TGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAAGAGTTGGTAGCTCTT GATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTITGCAA GCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGAT CTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTAAAGG GATTTTGGICATGAGATTATCAAAAAAGGATCTTCACCTAGATCCITTTA AATTA AAAATGAAGTTTAAATCAATCTAAAGTATATATGAGTAAACTT GGTCTGACAGTTAGAAAAACTCATCGAGCATCAAAATGAAACTGCAATT TATTCATATCAGGATTATCAATACCATATTTTTTGAAAAAGCCGTTTCTG TAATGAAGGAGAAAACTCACCGAGGCAGTTCCA TAGGATGGCAAGATC CTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATT AATTTCCCTCGTCAAAAAATAAGGTATCAAGTGAGAAATCACCATGA GTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCC AGACTTGTTC AACAGGCCAGCCATTACGCTCGTCATCAAAAACACTCGC ATCAACCAAACCGTTATTCATTCGTGATTGCGCTGAGCGAAACGAAA TACGCGATCGCTGTTAAAAAGGACAATTACAAACAGGAATCGAATGCCAA CCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATC AGGATATTCTTCTAATAACCTGGAATGCTGTTTTCCAGGGATCGCAGTG GTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTC GGAAGAGGCATAAAATCCGTCAGCCAGTTTAGTCTGACCATCTCATCTG TAACATCATTTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACCTCTGG CGCATCGGGCTTCCCATACAATCGATAGATTGTGCGACCTGATTGCCCG ACATTATCGCGAGCCATTTATAACCATATAAAATCAGCATCCATGTTGG AATTTAATCGCGGCCTAGAGCAAGACGTTTCCCGTTGAATATGGCTCAT ACTCTTCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCA TGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGG TTCCGCGCACATTTCCCGAAAAAGTGCCACCTGACGTCTAAGAAACCAT TATTATCATGACATTAACTATAAAAAATAAGGCGTATCAGGAGGCCCTTT TGTCTCGCGCGTTTCGGTGATGACGGTGAAAACTCTGACACATGCAGC TCCCGGAGACTGTACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGAC AAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGGGGCTGGC TTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGC GGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGC GCCATTCGCCATTACAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGC GGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAA GGCGATTAAGTTGGGTAAACGCCAGGGTTTCCAGTCAACGACGTTGTA AAACGACGGCCAGTGAATTGACGCGTATTGGGAT
--	--

[0302] As shown in FIG. 1, after transfecting the packing cell line, the GOI plasmid was used as a template to produce a single-stranded self-complementary DNA molecule, which can fold back upon itself to form a double-stranded hairpin structure with a mutated ITR in the middle, and two open-ended wild-type ITRs at the two ends, where the double-stranded region contained the transgene and regulatory sequences. The self-complementary genome was assembled as a single stranded DNA with capsid proteins synthesized in the packaging cells into a virion.

[0303] FIGs. 2A and 2B further illustrate examples of self-complementary AAV genome structures. As shown in FIG. 2A, the functional elements along the self-complementary AAV genome, including, from the 5' to 3' order, (a) a full-length AAV2 left (5') ITR sequence, (b) a CMV promoter including CMV enhancer and CMV core promoter sequences, (c) a chimeric intron sequence, (d) a NeuroD1 coding sequence, (e) a polyadenylation signal, (f) a AAV2 right (3') ITR sequence with the terminal resolution site (trs) deleted, and (g) a reverse complementary sequence of the above

(a) to (e) (element (g) is not shown in the bottom panel) . As shown in FIG. 2B, the functional elements along the self-complementary AAV genome, including, from the 5' to 3' order, (a) an AAV2 right (3') ITR sequence with the terminal resolution site (trs) deleted, (b) a CMV promoter including CMV enhancer and CMV core promoter sequences, (c) a chimeric intron sequence, (d) a NeuroD1 coding sequence, (e) a polyadenylation signal, (f) a full-length AAV2 left (5') ITR sequence, (g) a reverse complementary sequence of the above (b) to (f) (element (g) is not shown in the bottom panel) .

[0304] This self-complementary molecule forms a genome of <2.5kb, containing two wild-type left ITR sequences at both ends, and a mutant right ITR in the middle, upon packing into the capsid. Table 5.4 shows the sequences of functional fragments of the scAAV-NeuroD1 genome, and the full-length sequence of the genome.

6.1.2 Production of viral particles.

[0305] As illustrated in FIG. 1, AAV particles were produced by the triple transfection method using a HEK293 production cell line. Production and quality control of viral preparations for clinical use strictly followed cGMP standards. Particularly, HEK293 cells were seeded and expanded, and co-transfected with three plasmids: (1) a gene of interest (GOI) plasmid containing the transgene expression elements flanked by AAV ITRs; (2) a helper plasmid encoding adenovirus regions (VA, E2A and E4) that mediate AAV vector replication; and (3) a rep-cap packaging plasmid encoding the AAV capsid proteins (VP1, VP2, and VP3) through alternative splicing and initiation of translation, and AAV life cycle machinery Rep proteins (Rep78, Rep68, Rep52 and Rep40) through two promoters and alternative splicing.

[0306] Rep genes from AAV2 or AAV6 serotype were used for packaging AAV6 or AAV9 serotype virus, while capsid protein genes were serotype specific. Rep gene and encoded Rep protein sequences can be found in Table 6.1.2 (A) , SEQ ID NOS: 19-23. Cap gene and encoded capsid protein sequences for serotype 6 AAV virus can be found in Table 6.1.2 (A) , SEQ ID NOS: 24-27. Cap gene and encoded capsid protein sequences for serotype 9 AAV virus can be found in Table 6.1.2 (A) , SEQ ID NOS: 28-31. The full-length sequence of a Rep (serotype 2) /Cap (serotype 6) packaging plasmid used in the study can be found in Table 6.1.2 (B) , SEQ ID NO: 32. Helper plasmid sequences used in this study can be found in Table 6.1.2 (B) , SEQ ID NO: 33 and SEQ ID NO: 34.

[0307] After triple-transfection, the cells were harvested in lysis buffer at 48-72 hours post transfection. For clinical use, the viral particles were purified by affinity purification, followed by ultracentrifugation, and ion exchange filtration after treatment of Benzonase and clear out cell debris. For research use, viral particles were purified

by PEG as a preliminary purification step, followed by ultracentrifugation, and Ultra-filtration after treatment of Benzonase and clear out cell debris.

[0308] Final virus suspensions were produced after buffer exchange, concentration, and sterile filtration steps and were ready for in vitro or in vivo applications. The viral preparations were aliquoted before use.

Table 6.1.2 (A)

Rep / Cap sequences	
Coding sequence of AAV2 Rep proteins	ATGCCGGGGTTTTACGAGATTGTGATTAAAGGTCCCCAGCGACCTT GACGAGCATCTGCCCGGCATTCTGACAGCTTTGTGAACTGGGTG CCCGAGAAGGAATGGGAGTTGCCGCCAGATTCTGACATGGATCT GAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGC AGCGCGACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCCG GAGGCCCTTTTCTTTGTGCAATTTGAGAAGGGAGAGAGCTACTTC CACATGCACGTGCTCGTGGAAACCACCGGGGTGAAATCCATGGTT TTGGGACGTTTCTGAGTCAGATTTCGCGAAAAACTGATTCAGAGA ATTTACCGCGGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTC ACAAAGACCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGG ATGAGTGCTACATCCCCAATTACTTGCTCCCCAAAACCCAGCCTG AGCTCCAGTGGGCGTGGACTAATATGGAACAGTATTTAAGCGCCT GTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCGCAGCATCTGA CGCACGTGTCCGAGACGCAGGAGCAGAACAAAGAGAATCAGAAT CCCAATTCTGATGCGCCGGTGATCAGATCAAAAACCTTCAGCCAGG TACATGGAGCTGGTGGGTGGCTCGTGGACAAAGGGGATTACCTC GGAGAAGCAGTGGATCCAGGAGGACCAGGCCTCATACATCTCCT TCAATGCGGCCTCCAACTCGCGGTCCCAATCAAGGTGCCTTGG ACAATGCGGGAAAGATTATGAGCTTGAATAAACCGCCCCCGAC TACCTGGTGGGCCAGCAGCCCGTGGAGGACATTTCCAGCAATCG GATTTATAAAATTTTGGAACTAAACGGGTACGATCCCCAATATGC GGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCCGGCAAGAG GAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGACCA ACATCGCGGAGGCCATAGCCACACTGTGCCCTTCTACGGGTGCG TAAACTGGACCAATGAGAACTTTCCCTTCAACGACTGTGTCGACA AGATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTC GTGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGT GGACCAGAAATGCAAGTCTTCGGCCAGATAGACCCGACTCCCG TGATCGTCACCTCCAACACCAACATGTGCGCCGTGATTGACGGGA ACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATG TTCAAATTTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAG GTCACCAAGCAGGAAGTCAAAAGACTTTTTCGGTGGGCAAAAGGA TCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAGGGTG GAGCCAAGAAAAGACCCGCCCCAGTGACGCAGATATAAGTGAG CCCAAACGGGTGCGCGAGTCAGTTGCGCAGCCATCGACGTCAGA CGCGGAAGCTTCGATCAACTACGCAGACAGGTACCAAAAACAAAT

		GTTCTCGTCACGTGGGCATGAATCTGATGCTGTTCCCTGCAGAC AATGCGAGAGAATGAATCAGAATTCAAATATCTGCTTCACTCACG GACAGAAAAGACTGTTTAGAGTGCTTTCCCGTGTCAGAATCTCAAC CCGTTTCTGTCGTCAAAAAGGCGTATCAGAAACTGTGCTACATTC ATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCGATC TGGTCAATGTGGATTTGGATGACTGCATCTTTGAACAATAA
	Encoded AAV2 Rep78 amino acid sequence (SEQ ID NO:20)	MPGFYEIVIKVPSDLDEHLPGISDSFVNWVAEKEWELPPDSMDLNL IEQAPLTVAEKLQRDFLVQWRRVSKAPEALFFVQFEKGESYFHLHL VETTGVKSMVLGRFLSQIRDKLVTIYRGIEPTLPNWFAVTKTRNGA GGGNKVVDECYIPNYLLPKTQPELQWAWTNMEEYISACLNLAERKR LVAHDLTHVSQTQEQNKENLNPNSDAPVIRSKTSARYMELVGWL DRGITSEKQWQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKS APDYLVGPPADIKTNRIYRILELNGYDPAYAGSVFLGWAQKRFKG RNTIWLFGPATTGKTNIAEAIHAHVPFYGCNVNWTNENFPFND MVIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIV TSNTNMCAVIDGNSTTFEHQQPLQDRMFKFELTRRLEHDFGKVTKQ EVKEFFRWAQDHVTEVAHEFYVRKGGANKRPAPDDADKSEPKRAC PSVADPSTSDAEGAPVDFADRYQNKCSRHAGMLQMLFPCKTCERM NQNFNICFTHGTRDCSECFPGVSESQPVVRKRTYRKLCAIHLLGRA PEIACSACDLVNVDLDDCVSEQ
	Encoded AAV2 Rep68 amino acid sequence (SEQ ID NO:21)	MPGFYEIVIKVPSDLDGHLPGISDSFVNWVAEKEWELPPDSMDLNL IEQAPLTVAEKLQRDFLTEWRRVSKAPEALFFVQFEKGESYFHMHL VETTGVKSMVLGRFLSQIREKLIQRIYRGIEPTLPNWFAVTKTRNGA GGGNKVVDECYIPNYLLPKTQPELQWAWTNMEQYLSACLNLTERR RLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWL VDKGITSEKQWQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTK TAPDYLVGQPPVEDISSNRIYKILELNGYDPQYAASVFLGWATKKFG KRNTIWLFGPATTGKTNIAEAIHAHTVPFYGCNVNWTNENFPFND KMVIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVI VTSNTNMCAVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDHDFGKVTK QEVKDFFRWAKDHVVEVEHEFYVKKGGAKKRPA PSDADISEPKRV RESVAQPSTSDAEASINYADRLARGHSL
	Encoded AAV2 Rep52 amino acid sequence (SEQ ID NO:22)	MELVGWLVDKGITSEKQWQEDQASYISFNAASNSRSQIKAALDNA GKIMSLTKTAPDYLVGQPPVEDISSNRIYKILELNGYDPQYAASVFL GWATKKFGKRNTIWLFGPATTGKTNIAEAIHAHTVPFYGCNVNWTNEN FPFND CVDKMVIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSS AQIDPTPVIVTSNTNMCAVIDGNSTTFEHQQPLQDRMFKFELTRRLD HDFGKVTKQEVKDFFRWAKDHVVEVEHEFYVKKGGAKKRPA PSD ADISEPKRVRESVAQPSTSDAEASINYADRYQNKCSRHVGMNMLF PCRQCERMNQNSNICFTHGQKDCLECFVSESQPVSVVKKAYQKLC YIHHIMGKVPDACTACDLVNVDLDDCIFEQ
	Encoded AAV2 Rep40 amino acid sequence (SEQ ID NO:23)	MELVGWLVDKGITSEKQWQEDQASYISFNAASNSRSQIKAALDNA GKIMSLTKTAPDYLVGQPPVEDISSNRIYKILELNGYDPQYAASVFL GWATKKFGKRNTIWLFGPATTGKTNIAEAIHAHTVPFYGCNVNWTNEN FPFND CVDKMVIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSS AQIDPTPVIVTSNTNMCAVIDGNSTTFEHQQPLQDRMFKFELTRRLD

		HDFGKVTKEVVKDFFRWAKDHVVEVEHEFYVKKGGAKKRPAPSD ADISEPKRVRESVAQPSTSDAEASINYADRLARGHSL
Coding sequence of AAV6 capsid proteins (SEQ ID NO:24)		ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTC TCTGAGGGCATTCGCGAGTGGTGGGACTTGAAACCTGGAGCCCC GAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCGGGGT CTGGTGCTTCCTGGCTACAAGTACCTCGGACCCTTCAACGGACTC GACAAGGGGGAGCCCGTCAACGCGGCGGATGCAGCGGCCCTCGA GCACGACAAGGCCTACGACCAGCAGCTCAAAGCGGGTGACAATC CGTACCTGCGGTATAAACCACGCCGACGCCGAGTTTCAGGAGCGTC TGCAAGAAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCT TCCAGGCCAAAGAAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGG AAGGTGCTAAGACGGCTCCGGAAGAAACGTCGGTAGAGCAG TCGCCACAAGAGCCAGACTCCTCCTCGGGCATTGGCAAGACAGG CCAGCAGCCCCGCTAAAAAGAGACTCAATTTTGGTCAGACTGGCG ACTCAGAGTCAGTCCCCGACCCACAACCTCTCGGAGAACCTCCAG CAACCCCCGCTGCTGTGGGACCTACTACAATGGCTTCAGGCGGTG GCGCACCAATGGCAGACAATAACGAAGGCGCCGACGGAGTGGGT AATGCCTCAGGAAATTGGCATTGCGATTCCACATGGCTGGGCGAC AGAGTCATCACCACCAGCACCCGAACATGGGCCTTGCCACCTAT AACAAACCACCTCTACAAGCAAATCTCCAGTGCTTCAACGGGGGCC AGCAACGACAACCCTACTTCCGCTACAGCACCCCTGGGGGTAT TTTGATTTCAACAGATTCCACTGCCATTTCTCACCACCGTGACTGGC AGCGACTCATCAACAACAATTGGGGATTCCGGCCCAAGAGACTC AACTTCAAGCTCTTCAACATCCAAGTCAAGGAGGTACGACGAAT GATGGCGTCACGACCATCGCTAATAACCTTACCAGCACGGTTCAA GTCTTCTCGGACTCGGAGTACCAGTTGCCGTACGTCCTCGGCTCT GCGCACCAAGGGCTGCCTCCCTCCGTTCCCGGCGGACGTGTTCATG ATTCCGCAGTACGGCTACCTAACGCTCAACAATGGCAGCCAGGC AGTGGGACGGTCATCCTTTTACTGCCTGGAATATTTCCCATCGCA GATGCTGAGAACGGGCAATAACTTTACCTTCAGCTACACCTTCGA GGACGTGCCTTTCCACAGCAGCTACGCGCACAGCCAGAGCCTGG ACCGGCTGATGAATCCTCTCATCGACCAGTACCTGTATTACCTGA ACAGAACTCAGAATCAGTCCGGAAGTGCCCAAAACAAGGACTTG CTGTTTAGCCGGGGGTCTCCAGCTGGCATGTCTGTTACGCCCCAAA AACTGGCTACCTGGACCCTGTTACCGGCAGCAGCGCTTTCTAAA ACAAAAACAGACAACAACAACAGCAACTTTACCTGGACTGGTGC TTCAAAATATAACCTTAATGGGCGTGAATCTATAATCAACCCTGG CACTGCTATGGCCTCACACAAAGACGACAAAGACAAGTTCTTTCC CATGAGCGGTGTATGATTTTTTGGAAAGGAGAGCGCCGGAGCTTC AAACACTGCATTGGACAATGTATGATCACAGACGAAGAGGAAA TCAAAGCCACTAACCCCGTGGCCACCGAAAGATTTGGGACTGTG GCAGTCAATCTCCAGAGCAGCAGCACAGACCCTGCGACCGGAGA TGTGCATGTTATGGGAGCCTTACCTGGAATGGTGTGGCAAGACAG AGACGTATAACCTGCAGGGTCCCTATTTGGGCCAAAAATTCCTCACAC GGATGGACACTTTCACCCGTCTCCCTCTCATGGGCGGCTTTGGACT TAAGCACCCGCTCCTCAGATCCTCATCAAAAACACGCCTGTTC TGCGAATCCTCCGGCAGAGTTTTCGGCTACAAAGTTTGCTTCATT CATCACCCAGTATTCACAGGACAAGTGAGCGTGGAGATTGAAT GGGAGCTGCAGAAAGAAAACAGCAAAACGCTGGAATCCCGAAGTG CAGTATACATCTAACTATGCAAAATCTGCCAACGTTGATTTCACT

		GTGGACAACAATGGACTTTATACTGAGCCTCGCCCCATTGGCACC CGTTACCTCACCCGTCCTCTGTAA
	Encoded AAV6 VP1 amino acid sequence (SEQ ID NO:25)	MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRG LVLPGYKYLGPFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNP YLRYNHADADEFQERLQEDTSFGGNLGRAVFQAKKRVLEPFGLVVEEG AKTAPGKKRPVEQSPQEPDSSSGIGKTGQQPAKKRLNFGQTGDSSESV PDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGVGNASGN WHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHY FGYSTPWGYFDFNRFHCHFSRDPWQRLINNNWGFRPKRLNFKLFNI QVKEVTTNDGVTIANNLTSTVQVFSSEYQLPYVLGSAHQGCLPPF PADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNTFS YTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTQNQSGSAQNK DLLFSRGPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWT GASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGA SNTALDNVMITDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVH VMGALPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPMLGGFGLKH PPPQILIKNTVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKEN SKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL
	Encoded AAV6 VP2 amino acid sequence (SEQ ID NO:26)	MAPGKKRPVEQSPQEPDSSSGIGKTGQQPAKKRLNFGQTGDSSESVDP PQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGVGNASGNW HCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYF GYSTPWGYFDFNRFHCHFSRDPWQRLINNNWGFRPKRLNFKLFNIQ VKEVTTNDGVTIANNLTSTVQVFSSEYQLPYVLGSAHQGCLPPFP ADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNTFSY TFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTQNQSGSAQNKD LLFSRGPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTG ASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGAS NTALDNVMITDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHV MGALPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPMLGGFGLKHP PPQILIKNTVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKENS KRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL
	Encoded AAV6 VP3 amino acid sequence (SEQ ID NO:27)	MASGGGAPMADNNEGADGVGNASGNWHCDSTWLGDRVITTSTRT WALPTYNNHLYKQISSASTGASNDNHYFGYSTPWGYFDFNRFHCH SPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTIANNLT TVQVFSSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQ AVGRSSFYCLEYFPSQMLRTGNNTFSYTFEDVPFHSSYAHSQSLDR LMNPLIDQYLYLNRTQNQSGSAQNKDLLFSRGPAGMSVQPKNW LPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAM ASHKDDKDKFFPMSGVMIFGKESAGASNTALDNVMITDEEEIKATN PVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYL QGPIWAKIPHTDGHFHPSPMLGGFGLKHPPPQILIKNTVPANPPAEF SATKFASFITQYSTGQVSVEIEWELQKENSKRWNPEVQYTSNYAKSA NVDFTVDNNGLYTEPRPIGTRYLTRPL
	Coding sequence of AAV9 capsid proteins	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTT AGTGAAGGAATTCGCGAGTGGTGGGCTTTGAAACCTGGAGCCCC TCAACCCAAGGCAAATCAACAACATCAAGACAACGCTCGAGGTC

(SEQ ID NO:28)	TTGTGCTTCCGGGTTACAAATACCTTGGACCCGGCAACGGACTCG ACAAGGGGGAGCCGGTCAACGCAGCAGACGCGGGGCCCTCGAG CACGACAAGGCCTACGACCAGCAGCTCAAGGCCGGAGACAACCC GTACCTCAAGTACAACCACGCCGACGCCGAGTTCCAGGAGCGGC TCAAAGAAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCT TCCAGGCCAAAAAGAGGCTTCTTGAACCTCTTGGTCTGGTTGAGG AAGCGGCTAAGACGGCTCCTGGAAAAGAAGAGGCCTGTAGAGCAG TCTCCTCAGGAACCGGACTCCTCCGCGGGTATTGGCAAATCGGGT GCACAGCCCGCTAAAAAGAGACTCAATTTTCGGTCAGACTGGCGA CACAGAGTCAGTCCCAGACCCTCAACCAATCGGAGAACCTCCCG CAGCCCCCTCAGGTGTGGGATCTCTTACAATGGCTTCAGGTGGTG GCGCACCAAGTGGCAGACAATAACGAAGGTGCCGATGGAGTGGGT AGTTCCTCGGGAAATTGGCATTGCGATTCCCAATGGCTGGGGGAC AGAGTCATCACCACCAGCACCCGAACCTGGGGCCCTGCCACCTAC AACAAATCACCCTCTACAAGCAAATCTCCAACAGCACATCTGGAGG ATCTTCAAATGACAACGCCTACTTCGGCTACAGCACCCCTGGGG GTATTTTGACTTCAACAGATTCCACTGCCACTTCTCACCACGTGAC TGGCAGCGACTCATCAACAACAACCTGGGGATTCCGGCCTAAGCG ACTCAACTTCAAGCTCTTCAACATTCAGGTCAAGAGGTTACGGA CAACAATGGAGTCAAGACCATCGCCAATAACCTTACCAGCACGG TCCAGGTCTTCACGGACTCAGACTATCAGCTCCCGTACGTGCTCG GGTCCGGCTCAGGAGGGCTGCCCTCCCGCCGTTCACAGCGGACGTTT TCATGATTCTCAGTACGGGTATCTGACGCTTAATGATGGAAGCC AGGCCGTGGGTCGTTTCGTCTTTTACTGCCTGGAATATTTCCCGTC GCAAATGCTAAGAACGGGTAAACAACCTCCAGTTCAGCTACGAGTT TGAGAACGTACCTTTCCATAGCAGCTACGCTCACAGCCAAAGCCT GGACCGACTAATGAATCCACTCATCGACCAATACTTGTACTATCT CTCAAAGACTATTAACGGTTCTGGACAGAATCAACAAACGCTAA AATTCAGTGTGGCCGGACCCAGCAACATGGCTGTCCAGGGAAGA AACTACATACTTGGACCCAGCTACCGACAACAACGTGTCTCAACC ACITGTGACTCAAAAACAACAACAGCGAATTTTGTCTGGCCCTGGAGCT TCTTCTTGGGCTCTCAATGGACGTAATAGCTTGATGAATCCTGGA CCTGCTATGGCCAGCCACAAAGAAGGAGAGGACCGTTTCTTTCTT TTGTCTGGATCTTTAATTTTGGCAAACAAGGAACCTGGAAGAGAC AACGTGGATGCGGACAAAGTCATGATAACCAACGAAGAAGAAAT TAAAACCTACTAACCCGGTAGCAACGGAGTCCCTATGGACAAGTGG CCACAAAACCAAGAGTGCCCAAGCACAGGCGCAGACCGGCTGG GTTCAAAAACCAAGGAATACTTCCGGGTATGGTTTGGCAGGACAG AGATGTGTACCTGCAAGGACCCATTTGGGCCAAAATTCCTCACAC GGACGGCAACTTTACCCCTTCTCCGCTGATGGGAGGGTTTGGAAAT GAAGCACCCGCCTCCTCAGATCCTCATCAAAAACACACCTGTACC TGCGGATCCTCCAACGGCCTTCAACAAGGACAAGCTGAACTCTTT CATCACCCAGTATTCTACTGGCCAAGTCAGCGTGGAGATCGAGTG GGAGCTGCAGAAGGAAAACAGCAAGCGCTGGAACCCGGAGATCC AGTACACTTCCAACATAACAAGTCTAATAATGTTGAATTTGCTG TTAATACTGAAGGTGTATATAGTGAACCCCGCCCCATTGGCACCA GATACCTGACTCGTAATCTGTAA
Encoded AAV9 VP1 amino acid sequence	MAADGYLPDWLEDNLSEGIREWALKPGAPQPKANQQHQDNARG LVLPGYKYLGPNGLDKGEPVNAADAAALEHDKAYDQQLKAGDN PYLKYNHADADEFQERLKEDTSFGGNLGRAVFQAKKRILLEPLGLVEE

(SEQ ID NO:29)		AAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTES VPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGN WHCDSQWLGDREVITTSRTWALPTYNNHLYKQISNSTSGGSSNDNA YFGYSTPWGYFDENRFHCHFSRWDQRLINNNWGFRPKRLNFKLFN IQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAHEGCLPP FPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQF SYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQT LKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGA SSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDN VDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQ NQGILPGMVWQDRDVYLQGPWAKIPHTDGNFHPSPLMGGFGMKH PPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKEN SKRWNPEIQYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL
Encoded AAV9 VP2 amino acid sequence (SEQ ID NO:30)		APGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTESVPDP QPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGNWHC DSQWLGDREVITTSRTWALPTYNNHLYKQISNSTSGGSSNDNAYFG YSTPWGYFDENRFHCHFSRWDQRLINNNWGFRPKRLNFKLFNIQV KEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPA DVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYE FENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKF SVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSW ALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDA DKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNGGI LPGMVWQDRDVYLQGPWAKIPHTDGNFHPSPLMGGFGMKHPPPQI LIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL
Encoded AAV9 VP3 amino acid sequence (SEQ ID NO:31)		MASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDREVITTSRT WALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDENRFHCH FSRWDQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLT TSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDG SQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSL DRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNY IPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAM ASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNP VATESYGQVATNHQSAQAQAQTGWVQNGGILPGMVWQDRDVYLQ GPWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFN KDKLNSFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL

Table 6.1.2 (B)

Packaging and Helper Plasmids	
Rep (serotype 2) / Cap (serotype 6) Packaging Plasmid Sequence	CCGCCATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGAC CTTGACGAGCATCTGCCCGGCATTCTGACAGCTTTGTGAACTGGGT GGCCGAGAAGGAATGGGAGTTGCCGCCAGATTCTGACATGGATCTG AATCTGATTGAGCAGGCACCCCTGACCGTGCCGAGAAAGCTGCAGC GCGACTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCGGAGGC

7,344 bps (SEQ ID NO:32)	CCTTTTCTTTGTGCAATTTGAGAAGGGAGAGAGCTACTTCCACATGC ACGTGCTCGTGGAAACCACCGGGGTGAAATCCATGGTTTTGGGACG TTTCCTGAGTCAGATTCGCGAAAACTGATTTCAGAGAATTTACCGCG GGATCGAGCCGACTTTGCCAACTGGTTCGCGGTACAAAAGACCAG AAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTGCTACATC CCCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAGTGGGCGTG GACTAATATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACGGAGC GTAAACGGTTGGTGGCGCAGCATCTGACGCACGTGTCGCAGACGCA GGAGCAGAACAAAGAGAATCAGAATCCCAATTCGTGATGCGCCGGTG ATCAGATCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGC TCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGGAGGA CCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCC AAATCAAGGCTGCCTTGGACAATGCGGGAAGATTATGAGCCTGAC TAAAACCGCCCCGACTACCTGGTGGGCCAGCAGCCCGTGGAGGAC ATTTCCAGCAATCGGATTTATAAAATTTTGGAACTAAACGGGTACGA TCCCCAATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGT TCGGCAAGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGG GAAGACCAACATCGCGGAGGCCATAGCCCACACTGTGCCCTTCTAC GGGTGCGTAAACTGGACCAATGAGAACTTTCCCTTCAACGACTGTGT CGACAAGATGGTGTCTGGTGGGAGGAGGGGAAGATGACCGCCAA GGTCGTGGAGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTCCGC GTGGACCAAGAAATGCAAGTCCCTCGGCCAGATAGACCCGACTCCCG TGATCGTCACCTCCAACACCAACATGTGCGCCGTGATTGACGGGAAC TCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCA AATTTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACC AAGCAGGAAGTCAAAGACTTTTTCCGGTGGGCAAAGGATCACGTGG TTGAGGTGGAGCATGAATTCTACGTCAAAAAGGGTGGAGCCAAGAA AAGACCCGCCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTG CGCGAGTCAGTTGCGCAGCCATCGACGTACAGACGCGGAAGCTTCGA TCAACTACGCAGACAGGTACCAAAACAAATGTTCTCGTCACGTGGG CATGAATCTGATGCTGTTTCCCTGCAGACAATGCGAGAGAATGAATC AGAATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAGAG TGCTTTCCCGTGTGAGAATCTCAACCCGTTTCTGTGCTCAAAAAGGC GTATCAGAAACTGTGCTACATTCATCATATCATGGGAAAGGTGCCAG ACGCTTGCACTGCCTGCGATCTGGTCAATGTGGATTTGGATGACTGC ATCTTTGAACAATAAATGATTTAAATCAGGTATGGCTGCCGATGGTT ATCTTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCAATTCGCGAG TGGTGGGACTTGAAACCTGGAGCCCCGAAACCCAAAGCCAACCAGC AAAAGCAGGACGACGGCCGGGTCTGGTGTCTTCTGGCTACAAAGTA CCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCG GCCGATGCAGCGGCCCTCGAGCACGACAAGGCCCTACGACCAGCAGC TCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGCCGACGC CGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAAC CTCGGGCGAGCAGTCTTCCAGGCCAAGAAAGAGGGTTCTCGAACCTTT TGGTCTGGTTGAGGAAGGTGCTAAGACGGCTCCTGGAAAGAAACGT CCGGTAGAGCAGTCGCCACAAGAGCCAGACTCCTCCTCGGGCATTG GCAAGACAGGCCAGCAGCCCGCTAAAAAGAGACTCAATTTTGGTCA GACTGGCGACTCAGAGTCAGTCCCCGACCCACAACCTCTCGGAGAA CCTCCAGCAACCCCCGCTGCTGTGGGACCTACTACAATGGCTTCAGG CGGTGGCGCACCAATGGCAGACAATAACGAAGGCGCCGACGGAGTG GGTAATGCCTCAGGAAATTTGGCATTGCGATTCCACATGGCTGGGCG
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Helper Plasmid v.1 11,584 bps encoding VA, E4, E2A mediating AAV replication (SEQ ID NO:33)	GGTACCCAACTCCATGCTTAACAGTCCCCAGGTACAGCCCAACCCTGC GTCCGAACCAGGAACAGCTCTACAGCTTCCGAGCGCCACTCGCCC TACTTCCGCAGCCACAGTGGCGAGATTAGGAGCGCCACTTCTTTTGG TCACTTGAAAAACATGTAAAAATAATGTACTAGGAGACACTTTCAAT AAAGGCAAAATGTTTTTATTTGTACACTCTCGGGTGATTATTTACCCCC CACCCTTGCCGTCTGCGCCGTTTAAAAATCAAAGGGGTTCTGCCGCG CATCGCTATGCGCCACTGGCAGGGACACGTTGCGATACTGGTGTTTA GTGCTCCACTTAAACTCAGGCACAACCATCCGCGGCAGCTCGGTGA AGTTTTCACTCCACAGGCTGCGCACCATCACCAACGCGTTTACGAGG TCGGGCGCCGATATCTTGAAGTCGCAGTTGGGGCCTCCGCCCTGCGC GCGCGAGTTGCGATACACAGGGTTGCAGCACTGGAACACTATCAGC GCCGGGTGGTGCACGCTGCCAGCACGCTCTTGTGCGAGATCAGAT CCGCGTCCAGGTCTCCGCGTTGCTCAGGGCGAACGGAGTCAACTTT GGTAGCTGCCTTCCCAAAAAGGGTGCATGCCCAGGCTTTGAGTTGCA CTCGCACCGTAGTGGCATCAGAAGGTGACCGTGCCCGGTCTGGGCG TTAGGATACAGCGCCTGCATGAAAGCCTTGATCTGCTTAAAAGCCAC CTGAGCCTTTGCGCCTTCAGAGAAGAATGCGCGAAGACTTGCCG GAAAACTGATTGGCCGGACAGGCCGCGTCATGCACGCAGCACCTTG CGTCGGTGTTGGAGATCTGCACCACATTTCCGCCCCACCGGTTCTTC ACGATCTTGGCCTTGCTAGACTGCTCCTTCAGCGCGCGCTGCCCGTT TTCGCTCGTCACATCCATTTCAATCACGTGCTCCTTATTTATCATAAT GCTCCCGTGTAGACACTTAAGCTCGCCTTCGATCTCAGCGCAGCGGT GCAGCCACAACGCGCAGCCCGTGGGCTCGTGTTGTAGGTTACC TCTGCAAACGACTGCAGGTACGCCTGCAGGAATCGCCCCATCATCGT CACAAAGGTCTTGTGTTGCTGGTGAAGGTGAGCTGCAACCCGCGGTGCT CCTCGTTTAGCCAGGTCTTGCATACGGCCGCCAGAGCTTCCACTTGG TCAGGCAGTAGCTTGAAGTTTGGCTTTAGATCGTTATCCACGTGGTA CTTGTCCATCAACGCGCGCGCAGCCTCCATGCCCTTCTCCACGCGAG ACACGATCGGCAGGCTCAGCGGGTTTATCACCGTGCTTTCACCTTCC GCTTCACTGGACTCTTCCCTTTCCTCTTGCGTCCGCATACCCCGCGCC ACTGGGTGCTCTTCACTCAGCCGCCCGCACCGTGCGCTTACCTCCCTT GCCGTGCTTGATTAGCACCGGTGGGTTGCTGAAACCCACCATTTGTA GCGCCACATCTTCTCTTCTTCTTCTCGCTGTCCACGATCACCTCTGGGG ATGGCGGGCGCTCGGGCTTGGGAGAGGGGCGCTTCTTTTCTTTTGG GACGCAATGGCCAAATCCGCCGTCGAGGTGATGCCCGCGGGCTGG GTGTGCGCGGCACACAGCGCATCTTGTGACGAGTCTTCTTCGTCTCG GACTCGAGACGCCGCTCAGCCGCTTTTTTGGGGGCGCGCGGGGAG GCGGCGGCGACGGCGACGGGGACGACACGTCCTCCATGGTTGGTGG ACGTCGCGCCGCACCGCGTCCGCGCTCGGGGGTGGTTTCGCGCTGCT CCTCTTCCCGACTGGCCATTTCTTCTCTCTATAGGCAGAAAAAGATC ATGGAGTCAGTCGAGAAGGAGGACAGCCTAACCGCCCCCTTTGAGT TCGCCACCACCGCTCCACCGATGCCGCCAACGCGCTTACCACCTTC CCCGTCGAGGCACCCCGCTTGAGGAGGAGGAAGTGATTATCGAGC AGGACCCAGGTTTTGTAAAGCGAAGACGACGAGGATCGCTCAGTACC AACAGAGGATAAAAAAGCAAGACCAGGACGACGAGAGGCAAAACGA GGAACAAGTCGGGCGGGGGGACCAAGGCATGGCGACTACCTAGAT GTGGGAGACGACGTGCTGTTGAAGCATCTGCAGCGCCAGTGCGCCA TTATCTGCGACGCGTTGCAAGAGCGCAGCGATGTGCCCTCGCCATA GCGGATGTCAGCCTTGCTTACGAACGCCACCTGTTCTACCGCGCGT ACCCCCCAAACGCCAAGAAAACGGCACATGCGAGCCCCAACCCGCGC
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Helper Plasmid v.2	GGTACCCAACTCCATGCTTAACAGTCCCCAGGTACAGCCCACCCTGC
The sequence at 1-9280 (in upper case)	GTGCAACCAGGAACAGCTCTACAGCTTCCTGGAGCGCCACTCGCCC TACTTCCGCAGCCACAGTGCGCAGATTAGGAGCGCCACTTCTTTTGT TCACTTGAAAAACATGTAAAAATAATGTACTAGGAGACACTTTCAT

are from Adh5
including coding
sequences for E4,
E2a and VA; the
remaining sequence
(in lower case) is
plasmid backbone

(SEQ ID NO:34)

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6.1.3 Glioma cell cultures.

[0309] Glioblastoma cell lines (U87-Luc, U251-Luc and GL261-Luc) were seeded in 24 well plates (2×10^4 cells/well) and infected with ten-fold serial diluted rAAV (MOI: 10^6 to 10^3). Cell medium was replaced with fresh medium after 24hr. Cell viability was determined by CCK-8 assay (Cell Counting Kit-8, Dojindo Laboratoris) following manufacture manual 72 hrs post infection.

6.1.4 Animal care.

[0310] All animal procedures were performed using protocols approved by CRADL-SZ (Charles River Accelerator & Development Lab, Suzhou) Institutional Animal Care and Use Committee.

6.1.5 Experimental observation and data collection.

[0311] After inoculation of tumor cells, routine monitoring included general animal behavior, activity levels, food and water intake, weight fluctuations, eyes condition, coat appearance, and any abnormalities. Symptoms observed during the trial were recorded in the original data. Mice were weighed twice or three times a week throughout the duration of the experiment, depending on how close the animal were to the survival end point.

6.1.6 Section and immunostaining.

[0312] The mouse brain samples treated with NXL-004 or PBS were sectioned by RWD FS800 Cryostats, immune-stained with antibodies that specifically bind to NeuroD1,

GFP, neuronal cell marks including NeuN, GFAP, Ki67, human nuclei (HuNu) , DAPI etc. imaged and analyzed using Olympus VS200 fluorescent microscope.

6.1.7 Reverse transcriptase-quantitative PCR (RT-qPCR) .

- [0313] The brain tissues were harvested, and tumor sections were collected for mRNA analysis. RNA and DNA were extracted using AllPrep™ DNA/RNA Mini Kit (Qiagen) , and reverse transcription was performed using the Transcriptor™ first stand cDNA synthesis kit (Roche) . RT-qPCR was then performed using the QuantiNova™ SYBR Green PCR kit on the QuantStudio™ 6 Pro Real-Time PCR System.

6.1.8 Statistics.

- [0314] All statistical analyses were conducted using GraphPad Prism Software 9.3.1. The RT-qPCR data was analyzed using the Mann Whitney test to determine the significance of differences between experimental groups, with one-tailed or two-tailed p value calculated accordingly. Survival analysis was performed by Kaplan-Meier analyses, which generates a p-value to assess significance. The Kaplan-Meier test was performed using the log-rank test equivalent to the Mantel–Cox test.

6.2 Example 2: Serotype 6 Recombinant AAV Demonstrated Superior Efficiencies in Transducing Glioma Cells.

- [0315] To compare transduction efficiencies of different AAV serotypes and efficiencies of different promoter sequences in driving transgene expression in glioma cells, four different AAV particles were constructed according to the procedure described in Sections 6.1.1 and 6.1.2, including serotype 9 AAVs containing a single-stranded genome encoding a GFP protein under regulatory control of a GFAP promoter (ssAAV9-GFAP-GFP) , serotype 6 AAVs containing a single-stranded genome encoding a GFP protein under regulatory control of a GFAP promoter (ssAAV6-GFAP-GFP) , serotype 9 AAVs containing a self-complementary genome encoding GFP under regulatory control of a CMV promoter (scAAV9-CMV-GFP) , and serotype 6 AAVs containing a self-complementary genome encoding GFP under regulatory control of a CMV promoter (scAAV6-CMV-GFP) .
- [0316] Twenty-four hours after 7×10^3 of U87MG cells were plated in culture dish, the four different types of AAV viruses were each added to the cell cultures at multiplicity of infection (MOI) of 2×10^6 , respectively. Fluorescence of GFP were captured at each of 24h, 48h, 72h, 120h, and 168h after viral infection with same imaging parameters for all the groups (FIG. 4A) . The quantification results showed that the GFP fluorescence intensity emitted from cells treated with scAAV6-CMV-GFP was the highest among the four rAAVs tested (FIG. 4B) . Further, the GFP fluorescence intensity emitted from cells treated with rAAV encoding GFP under regulation of the CMV promoter was significantly higher than that under regulation of the GFAP promoter, and GFP

fluorescence intensity emitted from cells treated with the serotype 6 virus was significantly higher than that of the serotype 9 virus (FIG. 4B). These results demonstrate that scAAV6 particles are more efficient in transducing glioma cells than scAAV9, and the CMV promoter is superior in driving transgene expression in glioma cells than the GFAP promoter.

[0317] Furthermore, due to high heterogeneity between glioblastoma cells and also between other non-tumor cells belonging to the tumoral niche, a 3D glioblastoma sphere model was established to avoid possible limitations of the classical 2D cell culture systems for examining activities of the rAAVs. To establish the glioblastoma sphere model, glioblastoma cells (U87MG-Luc) were seeded on ultralow attachment 24 well plates at a concentration of 1,000 cells/mL in DMEM supplemented with 20 ng/mL of epidermal growth factor (EGF), 10 ng/mL of basic fibroblast growth factor, and 1× B27. Fresh medium was added every 3-4 days.

[0318] The cell culture was treated with scAAV6-CMV-GFP or scAAV9-CMV-GFP (MOI 1×10^4) at day 8 and observed under a fluorescence microscope at day 11. As shown in FIG. 4C, GFP fluorescence intensity emitted from cells treated with scAAV6-CMV-GFP was significantly higher than that of the scAAV9-CMV-GFP treated cells. These results again demonstrate that scAAV6 particles are superior and more efficient at transducing and driving transgene expression than scAAV9 in the U87MG-Luc sphere model.

6.3 Example 3: Self-complementary AAV6 Efficiently Transduced Glioma Cells in vivo

[0319] To examine in vivo transduction efficiency of scAAV6 in glioma cells, U87MG-Luc cells (5×10^5 cells per animals) were injected subcutaneously in BALB/c nude mice to generate the CDX tumor. When tumor volume is around 100 mm^3 in about 2 weeks post transplantation, 40 μl of scAAV6-CMV-GFP virus was injected into the tumor at 4 points with 10 μl per site. Three days later, the tumor samples were collected and sectioned with cryostat at 40 μm per slice. The sections were observed and imaged for GFP fluorescence. As shown in FIG. 5A, tumor cells at the injection site (circled by the dashed line) emitted strong green fluorescence, while cells outside the injection site was only stained blue (DAPI for nuclei). FIG. 5B is a close-up image of the injection site, showing a majority of tumor cells in the area were infected and expressed GFP. These results showed that intratumoral injection of scAAV6-CMV-GFP can efficiently infect tumor cells and express the transgene in the infected cells in vivo.

6.4 Example 4: scAAV6-NeuroD1 Efficiently Transduced and Killed Glioma Cells in vitro

- [0320] To examine the anti-tumor effect of NeuroD1 in glioma, scAAV6 virus encoding NeuroD1 were constructed using the methods described in Sections 6.1.1 and 6.1.2. The purified scAAV particles contained a DNA genome of SEQ ID NO: 16, which was packaged in a AAV serotype 6 capsid containing the VP1 protein (SEQ ID NO: 25) , VP2 protein (SEQ ID NO: 26) and VP3 protein (SEQ ID NO: 27) . This viral vector is referred to as scAAV6-NeuroD1 herein.
- [0321] To examine transduction efficiency of scAAV6-NeuroD1 in glioma cells, U87MG-Luc cells were seeded and treated with scAAV6-NeuroD1 or a negative control AAV6-NeuroD1-Null that carried a null mutant of NeuroD1 transgene without a start codon. Immunostaining of the U87MG-Luc cells for NeuroD1 (purple) and for nuclei marker DAPI (blue) was performed 8 days post treatment. As shown in FIG. 6 left panel, NeuroD1 expression was detected in a majority of cells treated with scAAV6-NeuroD1 (as indicated by the colocalization of the blue and purple signals) , whereas the right panel shows the lack of expression of NeuroD1 in cells treated with AAV6-NeuroD1-Null (as indicated by lack of purple staining) . These results demonstrate that scAAV6-NeuroD1 can efficiently infect glioma cells and express the encoded NeuroD1 in the infected glioma cells.
- [0322] Further, to examine the anti-tumor efficiency of scAAV6-NeuroD1, glioblastoma cell lines (U87MG-Luc, U251-Luc or GL261-Luc) were seeded in 24-well plates (2×10^4 cells/well) and infected with ten-fold serial diluted recombinant AAVs (MOI: 10^6 to 10^3) . Cell medium was replaced with fresh medium after 24hr. Cell viability was determined by CCK-8 assay (using Cell Counting Kit-8) 72 hours post infection. Viability of cells treated with PBS was set as 100%. As shown in FIG. 7, treatment with scAAV6-NeuroD1 reduced tumor cell viability in a dose-dependent manner.
- [0323] Next, anti-tumor efficacy of rAAVs were examined utilizing the 3D glioblastoma sphere model. Particularly, glioblastoma cells (U87MG-Luc) were seeded in 24 well plates (2×10^4 cells/well) and infected with either scAAV6-NeuroD1 or a serotype-comparative scAAV9-NeuroD1 viral preparation at MOI: 10^4 . The serotype-comparative scAAV9-NeuroD1 virus had the same scAAV genome encoding NeuroD1, but was packaged in a AAV9 capsid having the VP1 protein (SEQ ID NO: 29) , VP2 protein (SEQ ID NO: 30) , and VP3 protein (SEQ ID NO: 31) of serotype 9 virus instead.
- [0324] Treated U87MG-Luc cells were detached and reseeded on ultralow attachment 24-well plates at a concentration of 1,000 cells/mL in DMEM supplemented with 20 ng/mL of epidermal growth factor (EGF) , 10 ng/mL of basic fibroblast growth factor, and $1 \times B27$. Fresh medium was added every 3-4 days. Spheres were counted at 12 days after seeding. Counting of sphere formation numbers in U87MG-Luc cells showed scAAV6-NeuroD1 had significantly more inhibitory effect than the serotype

comparative scAAV9-NeuroD1 or PBS on in vitro sphere formation. FIG. 8 right panel shows average sphere formation numbers (from three duplicated experiments) in different groups treated with PBS, scAAV6-NeuroD1 or scAAV9-NeuroD1, respectively. Representative bright-field images of individual cell spheres are shown in the left panel.

[0325] Next, three types of tumor cell lines (BN2276, BN2338, BN9224) derived from three different glioma patients were infected with scAAV6-NeuroD1 in vitro (9-gradient multiplicity of infection). Tumor cell proliferations were assessed on day 7 post infection. The reading of untreated group was set as 100%. As shown in FIG. 9, at $\text{MOI} \geq 1 \times 10^5$ scAAV6-NeuroD1 was effective on inhibiting growth of all three tested patient-derived tumor cells. The inhibition had a dose threshold, and the inhibition efficiency was dose-dependent.

[0326] These results demonstrate a strong tumor killing effect of scAAV6-NeuroD1, much more superior than a serotype-comparative scAAV9-NeuroD1, suggesting therapeutic use of scAAV6-NeuroD1 in treating glioma.

6.5 Example 5: In vivo Therapeutic Efficacy of scAAV6-NeuroD1 in Treating Glioma

[0327] To examine in vivo therapeutic efficacy of scAAV6-NeuroD1 in treating glioma, an orthotopic cell-derived xenograft (CDX) model was established. Particularly, the U87-luc cells were transplanted into the striatum of BALB/c nude mouse brains using stereotactic intracranial injection. A total of 3×10^4 cells in 2 μl of PBS were injected at a speed of 0.5 $\mu\text{l}/\text{minute}$, with the injection coordinates being $\text{ap} = 0.50 \text{ mm}$, $\text{ml} = -2.0 \text{ mm}$, $\text{dv} = -3.50 \text{ mm}$ relative to bregma. On day 2, IVIS (in vivo imaging system) assays were performed and animals with luciferase signal that was higher than $1 \times 10^4 \text{ p/s/cm}^2/\text{sr}$ would be included in treatment or control group.

[0328] As shown in FIG. 3, at 3, 8 and 13 days post tumor cell transplantation (DPT), 4 μl of a formulation containing scAAV6-NeuroD1 virus at a titer of $1 \times 10^{13} \text{ vg/mL}$ in a phosphate-based buffer (e.g., containing potassium, potassium phosphate monobasic, sodium chloride, and sodium phosphate dibasic anhydrous) with poloxamer 188 was injected into the tumor at a speed of 0.5 $\mu\text{l}/\text{minute}$ (guided by same stereotactic injection coordinates as cell transplantation) at two depth with 2 μl each depth. The control group received the same volume of PBS, and the animals in both groups were handled similarly otherwise. On 14 DPT, 17 mice (8 animals from the PBS-treated control group and 9 animals from the scAAV6-NeuroD1 treatment group) were sacrificed, and brains were collected, sectioned, and stained for immunohistochemical, H&E staining, and RT-qPCR analysis for studying the in vivo efficacy and mechanism of scAAV6-NeuroD1. See specific animal number used in different experiments in results below. For assessment of survival efficacy, 40 animals (20 control and 20

treatment animals) were monitored until they reached the endpoint of life. Animals that had lost more than 20% of their body weight from their peak during the course of the experiment, or that had been determined by a veterinarian reaching survival endpoint, would to be euthanized.

[0329] FIG. 10A shows immunostaining of brain slices from animals sacrificed at 14 DPT. Human nuclei marker HuNu was stained in purple, neuronal marker NeuN was stained in green, and NeuroD1 was stained in red. All nuclei were stained in blue by DAPI staining. In the control group (upper panel), there was a dense DAPI staining area with obvious boundaries, where HuNu was also stained positive, indicating these were tumor cells. There was no NeuroD1 or NeuN staining in this area. In the treatment group (lower panel), the tumor area were HuNu stained positive, DAPI staining was similar to normal brain tissue nearby, and the tumor boundaries was not visible. High expression of NeuroD1 was detected in the tumor area, and the neuronal marker NeuN was also stained positive in this area, indicating transformation of tumor cells in the area into neuron or neuron like cells that express the NeuN marker. FIG. 10B shows the NeuroD1 expression in mRNA level of scAAV6-NeuroD1 infected tissue and tissue of negative control by RT-qPCR with housekeeping gene GAPDH as a reference.

[0330] As shown in FIG. 11, at 14 DPT, the average tumor size of animals in the treatment group was significantly smaller than that of the control group (quantitation shown in FIG. 11C). DAPI staining (blue, stained all nuclei) showed dense cell growth in the tumor area (FIG. 11A circled by dashed lines), which was also confirmed by histological studies based on H&E staining (FIG. 11B).

[0331] As shown in FIG. 12, on the second day after tumor transplantation in the glioma CDX model, in vivo IVIS imaging showed obvious signals in the brain in all animals. Animals were randomly assigned to treatment group or control group and test articles were administration on 3, 8 and 13 DPT. In vivo IVIS imaging on 8 and 14 DPT showed that the IVIS signal of the treatment group was significantly lower than that of the control group. It showed that treatment of scAAV6-NeuroD1 can significantly inhibit the tumor growth.

[0332] The nuclear protein, Ki67, is associated with cell proliferative activity, which may be an indicator of tumor aggressiveness. Accordingly, immunostaining of Ki67 was applied to assess cell proliferation in the glioma CDX model. As shown in FIG. 13, at 14 DPT, the expression of ki67 in the control group was higher as measured in both area and intensity as compared to those in the treatment group. Statistical analysis of the mouse brain slices of the treatment group and the control group showed significant differences in Ki67 signal. DAPI stained all nuclei.

- [0333] Expression level of Ki67 and NeuroD1 were measured by their mRNA levels using RT-qPCR analysis. As shown in FIG. 14, mRNA level of Ki67 in scAAV6-NeuroD1 treatment group was significantly lower than control (control n=7, scAAV6-NeuroD1-v1 n=6) , while NeuroD1 mRNA level in the treatment group was significantly higher than control (control, n=5; scAAV6-NeuroD1, n=6) . These data indicate that in vivo expression of NeuroD1 in glioma cells reduced proliferative activity of the glioma cells.
- [0334] Next, immunostaining analysis of microglia/macrophage marker Iba1 was performed to assess the inflammation ensuing the treatment. At 14 DPT, glioma CDX model mice received scAAV6-NeuroD1 or PBS (as a control) were sacrificed, and the brain slices were stained for the microglia/macrophage marker Iba1. DPAI stained for all nuclei. As shown in FIG. 15A, the Iba1 level elevated in the tumor area in treatment group compared to control, indicated that the innate immune response was upregulated in tumor area with treatment. As shown in FIG. 15B, quantitative analysis of Iba1 fluorescence showed significant difference of the two groups ($p = 0.0022$) . No change of Iba1 signal was found in contra-lateral hemisphere, suggesting there is no obvious whole-brain immune activation.
- [0335] The animals in treatment group and control group were weighed on multiple time points. The animals in control group started to show drastic body weight loss starting around 15 DPT and reached end of life quickly while treatment group stay stable and survive for much longer time.
- [0336] FIG. 16 shows the change of body weight of glioma CDX model mice received scAAV6-NeuroD1 or PBS (control) . As shown, body weight of the treatment group remained normal for an extended time as compared to the control group. FIG. 17 shows the median survival of control animals were 16 DPT (n=21) , while that of scAAV6-NeuroD1 treatment group reached 34 DPT (n=20) , ($p < 0.0001$) . Some animals in treatment group survived until experiment end point. These data indicate scAAV6-NeuroD1 was well tolerated by subjects suffering from glioma, and significantly extended their life span. FIG. 20 shows dose-dependent efficacy of scAAV6-NeuroD1 treatment on survival outcomes. This dose-dependent efficacy study demonstrates that scAAV6-NeuroD1 treatment significantly extended the median survival of the mice in a dose-dependent manner. The median survival times were 17 days for both the control and low-dose groups, 22.5 days for the mid-dose group, and 26 days for the high-dose group (n=9-10 per group, $p < 0.0001$) .
- [0337] To further demonstrate in vivo efficacy of scAAV6-NeuroD1 treatment, a patient-derived xenograft (PDX) model was established using tumor tissues isolated from real human patients. Particularly, NOD/SCID female mice were implanted with GBM tumor tissue with diameter of around 2-3mm subcutaneously. After the tumor volume

reached about 120 mm³, 50 µl of scAAV6-NeuroD1 with a titer of 1E13 vg/ml or PBS (control) were administered via intra-tumoral injection every 5 days. Tumor size was measured at regular intervals post-transplantation. scAAV6-NeuroD1 treatment significantly inhibited tumor growth compared to the control group (PBS) . FIG. 21 shows in vivo efficacy of scAAV6-NeuroD1 as measured in tumor growth in such PDX models of glioblastoma.

6.6 Example 6: Comparative Study

[0338] To compare in vivo therapeutic efficacies between different AAV serotypes and different transcriptional factors in the treatment of glioma, an orthotopic CDX GBM model was established using U87 cells as described above. Three different AAV vectors of AAV serotype 6 or 9 that encode NeuroD1 or Neurogenin2 (Ngn2) were prepared, namely scAAV6-NeuroD1 and its serotype comparative scAAV9-NeuroD1, as well as a comparative recombinant AAV9 comprising a self-complementary genome encoding Neurogenin2 (scAAV9-Ngn2) . For a direct comparison, the only difference between the scAAV9-Ngn2 vector and the scAAV9-NeuroD1 vector was their different coding sequences encoding NeuroD1 and Ngn2, respectively. As shown in FIG. 3, at 3, 8 and 13 DPT, 4 µl of a formulation containing scAAV6-NeuroD1 virus at a titer of 1×10^{13} vg/mL was injected into the tumor at a speed of 0.5 µl/minute (guided by same stereotactic injection coordinates as cell transplantation) at two depths with 2 µl each depth. A control group of mice received PBS injection instead. Survival rate of the treatment groups were monitored. As shown in FIG. 18, the median survival of the scAAV6-NeuroD1 treated group (24 days) was significantly longer than each of the control group (15 days) , and the scAAV9-Ngn2 treated group (17 days) and the scAAV9-NeuroD1 treated group (20 days) . These results confirmed that that AAV6 was a more suitable serotype than AAV9 for the treatment of glioma, and transcription factor NeuroD1 was more effective than Ngn2 for the treatment of glioma.

6.7 Example 7: Combination Therapy

[0339] An orthotopic CDX GBM model was generated using the U87MG-Luc cell line by injecting 2 µl U87MG-Luc cells (3×10^4 cells) into the striatum of the nude mice brain. The injection coordinates were AP = 0.50 mm, ML= -2.0mm, DV = -3.50mm. On 3, 8 and 13 dpt, scAAV6-NeuroD1 was injected at the same site in the striatum of the nude mouse brain. Temozolomide (TMZ) (5 mg/kg) were intraperitoneally administered 7, 8, and 9 dpt. As shown in FIG. 19, the median survival of control animals was 15.5 dpt (n=6) , while in the co-treatment group of scAAV6-NeuroD1 and TMZ, no animal died at the observation end point (86 dpt) , which was significantly improved compared to TMZ treatment alone (median survival = 28 days) or scAAV-NeuroD1 treatment alone (medial survival = 27 days) . In summary, the combination treatment of scAAV6-

NeuroD1 and TMZ showed significantly better efficacy than treatment with scAAV6-NeuroD1 or TMZ alone in mouse CDX GBM model.

6.8 Example 8: RNA-seq on GBM cells treated with scAAV6-NeuroD1 in vitro

[0340] Cultured GBM cells were treated with scAAV6-NeuroD1 or empty AAV6 capsids at 1E5 multiplicity of infection (MOI) . Three days post treatment, cells were collected and processed to extract total RNA. mRNA library was generated using 1 µl of total RNA with standard protocol. Then libraries with different indexes were multiplexed and loaded on an Illumina HiSeq/Illumina Novaseq/MGI2000 instrument for sequencing using a 2×150 paired-end (PE) configuration according to manufacturer's instructions. For transcriptome analysis, GSEq (v1.34.1) was used to identify Gene Ontology (GO) terms that annotate a list of enriched genes with a significant padj less or equal than 0.05.

[0341] As shown in Table 6.8 below, in vitro RNA-seq result of GBM cell lines treated with scAAV6-NeuroD1 or AAV9-NeuroD1 and control (PBS or empty viral capsid) showed that scAAV6-NeuroD1 induced significant transcriptome changes in the tested GBM lines U87 and 251, while AAV9-NeuroD1 induced less changes. Empty AAV capsid treatments have almost same result as PBS.

Table 6.8: Transcriptome change in cultured GBM cells with treatment of AAV-NeuroD1

GBM Cell Lines	Groups	Up-regulated Genes	Down-regulated Genes
U87	PBS vs 9-ND1	98	11
	PBS vs 6-ND1	2433	1558
	PBS vs 6-empty	0	0
	9-ND1 vs 6-ND1	1962	1346
	6-empty vs 6-ND1	2365	1459
U251	PBS vs 9-ND1	156	147
	PBS vs 6-ND1	1093	904
	PBS vs 6-empty	23	81
	9-ND1 vs 6-ND1	893	389
	6-empty vs 6-ND1	1211	581

[0342] U87 cells treated with scAAV6-NeuroD1 for 3 days showed broad down regulation of genes related to DNA replication, cell division and cell cycling indicating the inhibition of cell growth (FIGS. 22 and 23) . The result is consistent with the observed inhibition of cell proliferation and sphere formation of U87 cells by scAAV-NeuroD1 in culture. Result of U251 treated with scAAV6-NeuroD1 showed elevated gene expression related to neurodevelopment and neural activities (FIG. 24) , which indicated

the conversion possibly in progress. Because U87 and U251 have difference in genetic traits, they may respond to treatment differently while both responses slowed down the proliferation of tumor cells.

6.9 Example 9: Dose-Dependent Expression Study of NXL-004 in Normal Mouse Brain

[0343] To test the expression of NeuroD1 of the clinical product NXL-004 (scAAV6-NeuroD1), 2 μ l viral vector was injected to normal mouse brain in the striatum at 2E12 vg/ml, 6.6 E12 vg/ml or 2E13 vg/ml each. Brain tissue was collected 3 days post injection for immunostaining of NeuroD1. The result showed a dose dependent infection and NeuroD1 expression (FIG. 25).

6.10 Example 10: Clinical Database Analysis of NEUROD1 Expression in Glioma Patients

[0344] Analysis of patient databases, TCGA (The Cancer Genome Atlas), UCSC Xena, CGGA (Chinese Glioma Genome Atlas), revealed a significant correlation between high NEUROD1 expression and improved clinical outcomes. Specifically, high NEUROD1 expression was consistently associated with longer overall survival (OS) and progression-free interval (PFI) (FIGS. 26A and 26B). Further analysis indicated that NEUROD1 expression was significantly higher in IDH mutant (MT) gliomas than IDH wild-type (WT) glioma, and was inversely correlated with the WHO grade, with no significant difference on gender (FIGS. 27A and 27B). These findings were consistent across multiple datasets in CGGA (FIGS. 28A, 28B and 28C), further confirming that high NEUROD1 expression is associated with a better prognosis in glioma patients. Collectively, these results suggest that NeuroD1 expression is associated with lower tumor grade and better prognosis in glioma patients.

6.11 Example 11: Clinical studies

[0345] NXL-004 is being evaluated in an Investigator-Initiated Trial (IIT), which is an open-labeled, multi-center, dose-escalation exploratory clinical study evaluating the safety, tolerability, and preliminary efficacy of NXL-004 in the treatment of patients with malignant glioblastoma.

6.11.1 Patient population

[0346] Inclusion criteria:

1. Age ≥ 18 and ≤ 70 years old.
2. Histologically confirmed malignant glioma (WHO Grade 3-4).
3. Recurrence or progression after previous chemotherapy and/or radiation therapy, with at least one measurable lesion on contrast-enhanced MRI (≥ 1.0 cm, ≤ 5.5 cm).
4. Karnofsky Performance Status (KPS) score $\geq 60\%$.
5. Expected survival ≥ 3 months.

6. If patients are receiving corticosteroid treatment, the corticosteroid dose must have been stable for at least one week before treatment.

7. Male and female participants in the clinical study must agree to use effective contraception and continue it for 6 months after treatment.

8. Adequate organ function of liver, kidney and bone marrows: Neutrophil count $\geq 1500/\text{mm}^3$; Platelet count $\geq 100,000/\text{mm}^3$; Hemoglobin $\geq 9.0 \text{ g/dL}$; Serum creatinine ≤ 1.5 times the upper limit of normal (ULN); Bilirubin, Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT) ≤ 2.5 times ULN; Prothrombin time/international normalized ratio ≤ 1 .

6.11.2 Cohorts

[0347] Stratification of patients is based on whether the patients underwent tumor resection surgery. Stratification 1: Subjects who only underwent tissue biopsy and received intratumoral injection as the mode of administration:

Dose cohort 1: NXL-004 $2.0 \times 10^{12} \text{ vg/ml}$ x 1ml

Dose cohort 2: NXL-004 $6.6 \times 10^{12} \text{ vg/ml}$ x 1ml

Dose cohort 3: NXL-004 $2.0 \times 10^{13} \text{ vg/ml}$ x 1ml

Stratification 2: Subjects who underwent surgical resection and received intracavitary injection as

the mode of administration:

Dose cohort 4: NXL-004 $2.0 \times 10^{12} \text{ vg/ml}$ x 2ml

Dose cohort 5: NXL-004 $6.6 \times 10^{12} \text{ vg/ml}$ x 2ml

Dose cohort 6: NXL-004 $2.0 \times 10^{13} \text{ vg/ml}$ x 2ml

6.11.3 Administration Scheme

[0348] Stratification 1: Intratumoral Administration: After tissue biopsy, the initial administration of NXL-004 injection will be performed via stereotactic surgery for intratumoral injection. The total volume for a single administration is 1 ml. An Ommaya reservoir is placed on top of skull under scalp with the catheter inserted intratumorally. At least 15 days after the initial administration, if the patient demonstrates good safety and tolerability at the current dose level and, and continued treatment is expected to have clinical benefits according to the investigator's judgment, the patient receives two additional subsequent administrations. Each of these subsequent administrations is spaced 15 days apart, with a single administration volume of 1 mL, administered through the Ommaya reservoir.

[0349] Stratification 2: Intracavitary Administration: In a surgery, following the resection of a tumor, NXL-004 is injected into the surgical cavity with a single administration volume of 2 mL, and an Ommaya reservoir is placed on top of skull under scalp with catheter inserted in the surgical cavity. 30 days after the initial administration,

if the patient demonstrates good safety and tolerability at the current dose level, and continued treatment is expected to have clinical benefits according to the investigator's judgment, the patient receives two additional subsequent administrations. Each of these subsequent administrations will be spaced 30 days apart, with a single administration volume of 2 mL, administered through the Ommaya reservoir.

[0350] After completing the three intratumoral/intracavitary administrations, if imaging and clinical assessment suggest benefit and the criteria for continued treatment are met, the patient can receive additional administrations until no longer meeting the criteria.

[0351] Dosing Regimens

[0352] The dosing regimen in the various arms of clinical trials are provided below.

Arms	Intervention / Treatment
Stratification 1, Cohort 1 2.0×10^{12} vg NXL-004	Subject receives 1mL solution containing 2.0×10^{12} vg/mL NXL-004 injected intratumorally once every 15 days, for once, twice, or at least three times
Stratification 1, Cohort 2 6.6×10^{12} vg NXL-004	Subject receives 1mL solution containing 6.6×10^{12} vg/mL NXL-004 injected intratumorally once every 15 days, for once, twice, or at least three times
Stratification 1, Cohort 3 2.0×10^{13} vg NXL-004	Subject receives 1mL solution containing 2.0×10^{13} vg/mL NXL-004 injected intratumorally once every 15 days, for once, twice, or at least three times
Stratification 2, Cohort 4 4.0×10^{12} vg NXL-004	Subject receives 2mL solution containing 2.0×10^{12} vg/mL NXL-004 injected into the surgical cavity once every 15 days, for once, twice, or at least three times
Stratification 2, Cohort 5 1.32×10^{13} vg NXL-004	Subject receives 2mL solution containing 6.6×10^{12} vg/mL NXL-004 injected into the surgical cavity once every 15 days, for once, twice, or at least three times
Stratification 2, Cohort 6 4.0×10^{13} vg NXL-004	Subject receives 2mL solution containing 2.0×10^{13} vg/mL NXL-004 injected into the surgical cavity once every 15 days, for once, twice, or at least three times

6.11.4 Primary Outcome Measures:

[0353] Safety and tolerance within 180 days after intratumoral or intracavitary injection of NXL-004 based on incidences of adverse events (AE) and serious adverse events (SAE) assessed according to CTCAE 5.0.

6.11.5 Secondary Outcome Measures:

[0354] Efficacy of NXL-004 in treating malignant gliomas assessed according to

1. Objective response rate (ORR: PR+CR, iRANO criteria) ;
2. Overall Survival (OS) ;
3. Progression-Free Survival (PFS) ;
4. 6-Month PFS Rate;
5. 6-Month and 1-Year Survival Rates.

Claims

- [Claim 1] A single-stranded self-complementary nucleic acid molecule encoding a NeuroD1 polypeptide, wherein the nucleic acid molecule comprises an expression cassette comprising a coding sequence and one or more regulatory elements operably linked to the coding sequence, wherein the NeuroD1 polypeptide comprises an amino acid sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO: 1.
- [Claim 2] The nucleic acid molecule of claim 1, wherein the NeuroD1 polypeptide comprises an amino acid sequence set forth in SEQ ID NO: 4.
- [Claim 3] The nucleic acid molecule of claim 2, wherein the coding sequence comprises the nucleotide sequence set forth in SEQ ID NO: 5 or a codon-optimized variant thereof.
- [Claim 4] The nucleic acid molecule of any one of claims 1 to 3, wherein the one or more transcription regulatory elements comprise a chimeric intron.
- [Claim 5] The nucleic acid molecule of claim 4, wherein the chimeric intron comprises the sequence set forth in SEQ ID NO: 8.
- [Claim 6] The nucleic acid molecule of any one of claims 1 to 5, wherein the one or more transcription regulatory elements further comprise a CMV promoter comprising the sequence set forth in SEQ ID NO: 7.
- [Claim 7] The nucleic acid molecule of any one of claims 1 to 6, wherein the one or more transcription regulatory elements further comprises a CMV enhancer comprising the sequence set forth in SEQ ID NO: 6.
- [Claim 8] The nucleic acid molecule of any one of claims 1 to 7, wherein the one or more transcription regulatory elements further comprise a polyadenylation (poly-A) signal comprising the sequence set forth in SEQ ID NO: 9.
- [Claim 9] The nucleic acid molecule of any one of claim 1 to 8, further comprises a pair of terminal reverse-complementary fragments each located on one end of the nucleic acid molecule, an anti-sense region that is reverse-complementary to the expression cassette, and a central reverse-complementary fragment that connects the expression cassette and anti-sense region, wherein each of the pair of terminal reverse-complementary fragments and the central reverse-complementary fragment is capable of forming a stem-loop structure.

- [Claim 10] The nucleic acid molecule of claim 9, wherein at least one of the pair of terminal reverse-complementary fragments comprises a first inverted terminal repeat (ITR) of a first AAV genome.
- [Claim 11] The nucleic acid molecule of claim 10, wherein the first ITR is the 5' ITR of the first AAV genome.
- [Claim 12] The nucleic acid molecule of claims 10 or 11, wherein the first AAV genome is the AAV2 genome.
- [Claim 13] The nucleic acid molecule of claim 9, wherein the pair of terminal reverse-complementary fragments comprises the sequence set forth in SEQ ID NO: 13 and SEQ ID NO: 36, respectively.
- [Claim 14] The nucleic acid molecule of any one of claims 9 to 13, wherein the central reverse-complementary fragment comprises a second ITR of a second AAV genome.
- [Claim 15] The nucleic acid molecule of claim 14, wherein the central reverse-complementary fragment comprises the 3' ITR of the second AAV genome, wherein the 3' ITR is mutated to disrupt a terminal resolution site (trs) .
- [Claim 16] The nucleic acid molecule of claim 14 or 15, wherein the second AAV genomes is the AAV2 genome.
- [Claim 17] The nucleic acid molecule of any one of claims 9 to 13, wherein the central reverse-complementary fragment comprises the sequence set forth in SEQ ID NO: 14.
- [Claim 18] The nucleic acid molecule of claim 1, comprising the sequence set forth in SEQ ID NO: 16.
- [Claim 19] The nucleic acid molecule of any one of claims 1 to 18, wherein the nucleic acid molecule is DNA.
- [Claim 20] A single-stranded self-complementary DNA molecule consists of the sequence set forth in SEQ ID NO: 16.
- [Claim 21] A recombinant adeno-associated virus (rAAV) comprising a self-complementary genome, wherein the self-complementary genome comprises the DNA molecule of any one of claims 1 to 20.
- [Claim 22] The recombinant AAV of claim 21, wherein the recombinant AAV comprises a AAV serotype 6 (AAV6) capsid.
- [Claim 23] The recombinant AAV of claim 21 or 22, wherein the AAV6 capsid comprises capsid proteins selected from the group of AAV6 VP1 polypeptides, AAV6 VP2 polypeptides and AAV6 VP3 polypeptides.

- [Claim 24] The recombinant AAV of claim 22, wherein the AAV6 capsid comprises AAV6 VP1 comprising the amino acid sequence set forth in SEQ ID NO: 25.
- [Claim 25] The recombinant AAV of claim 22 or 24, wherein the AAV6 capsid further comprises AAV6 VP2 comprising the amino acid sequence set forth in SEQ ID NO: 26.
- [Claim 26] The recombinant AAV of any one of claims 22, 24 or 25, wherein the AAV6 capsid further comprises AAV6 VP3 comprising the amino acid sequence set forth in SEQ ID NO: 27.
- [Claim 27] A pharmaceutical composition comprising the recombinant AAV of any one of claims 21 to 26, wherein the pharmaceutical composition further comprises:
- (a) potassium chloride,
 - (b) potassium phosphate monobasic,
 - (c) sodium chloride,
 - (d) sodium phosphate dibasic anhydrous, and
 - (e) poloxamer 188, polysorbate 20, or polysorbate 80.
- [Claim 28] A pharmaceutical composition consists of:
- (a) a recombinant adeno-associated virus (AAV) ,
 - (b) sodium chloride at a concentration of about 180 mM;
 - (c) sodium phosphate at a concentration of about 10 mM; and
 - (d) poloxamer 188 at a concentration of about 0.001%weight/volume (0.01 g/L) ; and
- wherein the pH of the pharmaceutical composition is about 7.3.
- [Claim 29] A pharmaceutical composition consists of:
- (a) a recombinant adeno-associated virus (AAV) ,
 - (b) sodium chloride at a concentration of about 200 mM;
 - (c) magnesium chloride at a concentration of about 1 mM;
 - (d) Tris hydrochloride at a concentration of about 20 mM, and
 - (e) poloxamer 188 at a concentration of about 0.005%weight/volume (0.05 g/L) ; and
- wherein the pH of the pharmaceutical composition is about 8.0.
- [Claim 30] A pharmaceutical composition consists of:
- (a) a recombinant adeno-associated virus (AAV) ,
 - (b) sodium chloride at a concentration of about 150 mM;
 - (c) calcium chloride at a concentration of about 1.4 mM;
 - (d) magnesium chloride at a concentration of about 0.8 mM,
 - (e) sodium phosphate at a concentration of about 1 mM, and

(f) poloxamer 188 at a concentration of about 0.001%weight/volume (0.01 g/L) ; and

wherein the pH of the pharmaceutical composition is about 7.4.

[Claim 31] The pharmaceutical composition of any one of claims 28 to 30, wherein recombinant AAV is the recombinant AAV of any one of claims 21 to 26.

[Claim 32] The pharmaceutical composition of any one of claims 27 to 31, wherein the recombinant AAV is AAV6.

[Claim 33] The pharmaceutical composition of any one of claims 27 to 32, wherein a vector genome concentration of the recombinant AAV in the pharmaceutical composition is in the range of about 5×10^{11} to about 1×10^{14} viral genomes per mL (vg/mL) ; optionally, wherein the vector genome concentration is about 1×10^{12} vg/mL, about 1.5×10^{12} vg/mL, about 2×10^{12} vg/mL, about 2.5×10^{12} vg/mL, about 3×10^{12} vg/mL, about 3.5×10^{12} vg/mL, about 4×10^{12} vg/mL, about 4.5×10^{12} vg/mL, about 5×10^{12} vg/mL, about 5.5×10^{12} vg/mL, about 6×10^{12} vg/mL, about 6.5×10^{12} vg/mL, about 6.6×10^{12} vg/mL, about 7×10^{12} vg/mL, about 7.5×10^{12} vg/mL, about 8×10^{12} vg/mL, about 8.5×10^{12} vg/mL, about 9×10^{12} vg/mL, about 9.5×10^{12} vg/mL, about 1×10^{13} vg/mL, about 1.5×10^{13} vg/mL, about 2×10^{13} vg/mL, about 2.5×10^{13} vg/mL, about 3×10^{13} vg/mL, about 3.5×10^{13} vg/mL, about 4×10^{13} vg/mL, about 4.5×10^{13} vg/mL, about 5×10^{13} vg/mL, about 5.5×10^{13} vg/mL, about 6×10^{13} vg/mL, about 6.5×10^{13} vg/mL, about 7×10^{13} vg/mL, about 7.5×10^{13} vg/mL, about 8×10^{13} vg/mL, about 8.5×10^{13} vg/mL, about 9×10^{13} vg/mL, about 9.5×10^{13} vg/mL, or about 1×10^{14} vg/mL.

[Claim 34] A method for treating glioma, comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising a recombinant AAV, wherein the recombinant AAV comprises a self-complementary genome comprising a transgene encoding a NeuroD1 polypeptide.

[Claim 35] The method of claim 34, wherein the self-complementary genome is a single-stranded DNA molecule comprising a pair of terminal reverse-complementary fragments each located on one end of the DNA molecule, an expression cassette comprising a coding sequence of the transgene, an anti-sense region that is reverse complementary to the expression cassette, and a central reverse-complementary fragment that connects the expression cassette and anti-sense region; and wherein

each of the pair of terminal reverse-complementary fragments and the central reverse-complementary fragment is capable of forming a stem-loop structure.

- [Claim 36] The method of claim 34 or 35, wherein the NeuroD1 polypeptide comprises an amino acid sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO: 1.
- [Claim 37] The method of claim 34 or 35, wherein the coding sequence of the transgene comprises the nucleotide sequence set forth in SEQ ID NO: 5 or a codon-optimized version thereof.
- [Claim 38] The method of any one of claims 35 to 37, wherein the expression cassette further comprises one or more transcription regulatory elements operably linked to the coding sequence of the transgene.
- [Claim 39] The method of claim 38, wherein the one or more transcription regulatory elements comprise a chimeric intron.
- [Claim 40] The method of claim 39, wherein the chimeric intron comprises the sequence of SEQ ID NO: 8.
- [Claim 41] The method of any one of claims 38 to 40, wherein the one or more transcription regulatory elements further comprise a CMV promoter comprising the sequence of SEQ ID NO: 7; optionally the one or more transcription regulatory element further comprises a CMV enhancer comprising the sequence of SEQ ID NO: 6.
- [Claim 42] The method of any one of claims 38 to 41, wherein the one or more transcription regulatory elements further comprise a polyadenylation (poly-A) signal comprising the sequence of SEQ ID NO: 9.
- [Claim 43] The method of any one of claims 35 to 42, wherein at least one of the pair of terminal reverse-complementary fragments comprises a first inverted terminal repeat (ITR) of a first AAV genome; optionally wherein the first ITR is the 5' ITR of the first AAV genome.
- [Claim 44] The method of claim 43, wherein the first AAV genome is the AAV2 genome.
- [Claim 45] The method of any one of claims 35 to 42, wherein the pair of terminal reverse-complementary fragments are reverse complementary to one another.
- [Claim 46] The method of any one of claims 35 to 42, wherein the pair of terminal reverse-complementary fragments comprises the sequence set forth in SEQ ID NO: 13 and SEQ ID NO: 36, respectively.

- [Claim 47] The method of any one of claims 35 to 46, wherein the central reverse-complementary fragment comprises a second ITR of a second AAV genome.
- [Claim 48] The method of claim 47, wherein the central reverse-complementary fragment comprises the 3' ITR of the second AAV genome, wherein the 3' ITR is mutated to disrupt a terminal resolution site (trs) .
- [Claim 49] The method of claim 47 or 48, wherein the second AAV genomes is the AAV2 genome.
- [Claim 50] The method of any one of claims 35 to 46, wherein the central reverse-complementary fragment comprises the sequence set forth in SEQ ID NO: 14.
- [Claim 51] The method of claim 34, wherein the self-complementary genome of the recombinant AAV comprises the sequence set forth in SEQ ID NO: 16.
- [Claim 52] The method of any one of claims 34 to 51, wherein the recombinant AAV comprises an AAV serotype 6 (AAV6) capsid.
- [Claim 53] The method of claim 52, wherein the AAV6 capsid comprises capsid proteins selected from the group of AAV6 VP1 polypeptides, AAV6 VP2 polypeptides and AAV6 VP3 polypeptides.
- [Claim 54] The method of claim 52, wherein the AAV6 capsid comprises AAV6 VP1 comprising the amino acid sequence set forth in SEQ ID NO: 25.
- [Claim 55] The method of claim 52 or 54, wherein the AAV6 capsid further comprises AAV6 VP2 comprising the amino acid sequence set forth in SEQ ID NO: 26.
- [Claim 56] The method of any one of claims 52, 54 and 55, wherein the AAV6 capsid further comprises AAV6 VP3 comprising the amino acid sequence set forth in SEQ ID NO: 27.
- [Claim 57] The method of any one of claims 34 to 56, wherein the pharmaceutical composition is administered to the subject intratumorally.
- [Claim 58] The method of any one of claims 34 to 56, wherein the subject has a surgical cavity created by removing a glioma surgically, and wherein the pharmaceutical composition is administered to the surgical cavity or surrounding tissue.
- [Claim 59] The method of any one of claims 34 to 58, wherein the pharmaceutical composition comprises from about 5×10^{11} to about 1×10^{14} viral genomes (vg) of the recombinant AAV.

- [Claim 60] The method of any one of claims 34 to 58, wherein the pharmaceutical composition comprises about 2×10^{12} vg of the recombinant AAV.
- [Claim 61] The method of any one of claims 34 to 58, wherein the pharmaceutical composition comprises about 6.6×10^{12} vg of the recombinant AAV.
- [Claim 62] The method of any one of claims 34 to 58, wherein the pharmaceutical composition comprises about 2×10^{13} vg of the recombinant AAV.
- [Claim 63] The method of any one of claims 34 to 58, wherein the pharmaceutical composition comprises about 4×10^{12} vg of the recombinant AAV.
- [Claim 64] The method of any one of claims 34 to 58, wherein the pharmaceutical composition comprises about 1.32×10^{13} vg of the recombinant AAV.
- [Claim 65] The method of any one of claims 34 to 58, wherein the pharmaceutical composition comprises about 4×10^{13} vg of the recombinant AAV.
- [Claim 66] The method of any one of claims 34 to 56, wherein the subject is administered intratumorally the pharmaceutical composition comprising about 2×10^{12} vg of the recombinant AAV once every 15 days for once, twice or at least three times; optionally wherein for each administration the subject is administered about 1 mL of the pharmaceutical composition comprising about 2×10^{12} vg/mL of the recombinant AAV intratumorally; optionally wherein the pharmaceutical composition is administered through a Ommaya reservoir.
- [Claim 67] The method of any one of claims 34 to 56, wherein the subject is administered intratumorally the pharmaceutical composition comprising about 6.6×10^{12} vg of the recombinant AAV once every 15 days for once, twice or at least three times; optionally wherein for each administration the subject is administered about 1 mL of the pharmaceutical composition comprising about 6.6×10^{12} vg/mL of the recombinant AAV intratumorally; optionally wherein the pharmaceutical composition is administered through a Ommaya reservoir.
- [Claim 68] The method of any one of claims 34 to 56, wherein the subject is administered intratumorally the pharmaceutical composition comprising about 2×10^{13} vg of the recombinant AAV once every 15 days for once, twice or at least three times; optionally wherein for each administration the subject is administered about 1 mL of the pharmaceutical composition comprising about 2×10^{13} vg/mL of the recombinant AAV intratumorally; optionally wherein the pharmaceutical composition is administered through a Ommaya reservoir.

- [Claim 69] The method of any one of claims 34 to 56, wherein the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 4×10^{12} vg of the recombinant AAV to the surgical cavity once every 30 days for once, twice or at least three times; optionally wherein for each administration the subject is administered about 2 mL of the pharmaceutical composition comprising about 2×10^{12} vg/mL of the recombinant AAV to the surgical cavity; optionally wherein the pharmaceutical composition is administered through a Ommaya reservoir.
- [Claim 70] The method of any one of claims 34 to 56, wherein the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 1.32×10^{13} vg of the recombinant AAV to the surgical cavity once every 30 days for once, twice or at least three times; optionally wherein for each administration the subject is administered about 2 mL of the pharmaceutical composition comprising about 6.6×10^{12} vg/mL of the recombinant AAV to the surgical cavity; optionally wherein the pharmaceutical composition is administered through a Ommaya reservoir.
- [Claim 71] The method of any one of claims 34 to 56, wherein the subject has a surgical cavity created by removing a glioma surgically, and wherein the subject is administered the pharmaceutical composition comprising about 4×10^{13} vg of the recombinant AAV to the surgical cavity once every 30 days for once, twice or at least three times; optionally wherein for each administration the subject is administered about 2 mL of the pharmaceutical composition comprising about 2×10^{13} vg/mL of the recombinant AAV to the surgical cavity; optionally wherein the pharmaceutical composition is administered through a Ommaya reservoir.
- [Claim 72] The method of any one of claims 34 to 71, wherein the pharmaceutical composition further comprises:
- (a) potassium chloride,
 - (b) potassium phosphate monobasic,
 - (c) sodium chloride,
 - (d) sodium phosphate dibasic anhydrous, and
 - (e) poloxamer 188, polysorbate 20, or polysorbate 80.
- [Claim 73] The method of any one of claims 34 to 71, wherein the pharmaceutical composition further comprises:
- (a) sodium chloride at a concentration of about 180 mM;

- (b) sodium phosphate at a concentration of about 10 mM; and
(c) poloxamer 188 at a concentration of about 0.001%weight/volume (0.01 g/L) ; and
wherein the pH of the pharmaceutical composition is about 7.3.
- [Claim 74] The method of any one of claims 34 to 71, wherein the pharmaceutical composition further comprises:
(a) sodium chloride at a concentration of about 200 mM;
(b) magnesium chloride at a concentration of about 1 mM;
(c) Tris hydrochloride at a concentration of about 20 mM, and
(d) poloxamer 188 at a concentration of about 0.005%weight/volume (0.05 g/L) ; and
wherein the pH of the pharmaceutical composition is about 8.0.
- [Claim 75] The method of any one of claims 34 to 71, wherein the pharmaceutical composition further comprises:
(a) sodium chloride at a concentration of about 150 mM;
(b) calcium chloride at a concentration of about 1.4 mM;
(c) magnesium chloride at a concentration of about 0.8 mM,
(d) sodium phosphate at a concentration of about 1 mM, and
(e) poloxamer 188 at a concentration of about 0.001%weight/volume (0.01 g/L) ; and
wherein the pH of the pharmaceutical composition is about 7.4.
- [Claim 76] The method of any one of claims 34 to 75, wherein the glioma is selected from adult-type diffuse gliomas; astrocytoma, IDH-mutant; oligodendroglioma, IDH-mutant, and 1p/19q-codeleted; glioblastoma, IDH-wildtype; pediatric-type diffuse low-grade gliomas; diffuse astrocytoma, MYB-or MYBL1-altered; angiocentric glioma; polymorphous low-grade neuroepithelial tumor of the young; diffuse low-grade glioma, MAPK pathway-altered; pediatric-type diffuse high-grade gliomas; diffuse midline glioma, H3 K27-altered; diffuse hemispheric glioma, H3 G34-mutant; diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype; infant-type hemispheric glioma; circumscribed astrocytic gliomas; pilocytic astrocytoma; high-grade astrocytoma with piloid features; pleomorphic xanthoastrocytoma; subependymal giant cell astrocytoma; chordoid glioma; astroblastoma, MN1-altered; ganglioglioma; and esmoplastic infantile ganglioglioma.
- [Claim 77] The method of any one of claims 34 to 75, wherein the glioma is selected from oligodendroglioma, IDH mutant, and 1p/19q-codeleted

- (WHO grade 3) ; astrocytoma, IDH mutant (WHO grade 3, 4) , and glioblastoma, IDH wildtype (WHO grade 4) .
- [Claim 78] The method of any one of claims 34 to 75, wherein the glioma is selected from diffuse midline glioma, H3 K27-altered; diffuse hemispheric glioma, H3 G34-mutant; diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype; and infant-type hemispheric glioma.
- [Claim 79] The method of any one of claims 34 to 78, wherein the subject is treatment^{naïve} for glioma.
- [Claim 80] The method of any one of claims 34 to 78, wherein the subject has received a previous therapy for treating glioma, and the glioma is refractory to or relapsed from the previous therapy.
- [Claim 81] The method of any one of claims 34 to 80, wherein the previous therapy is radiation therapy or chemotherapy.
- [Claim 82] The method of any one of claims 34 to 81, wherein the subject is a human.
- [Claim 83] The method of any one of claims 34 to 82, wherein upon administering the pharmaceutical composition, the NeuroD1 polypeptide is expressed by a population of glioma cells.
- [Claim 84] The method of claim 83, wherein proliferative activity in the population of glioma cells reduces; optionally wherein at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95% of the glioma cells in the population stop proliferation; optionally wherein proliferative activity in the population of glioma cells reduces in less than 14 days after the administering of the pharmaceutical composition.
- [Claim 85] The method of claim 83, wherein the population of glioma cells exhibit one or more neuronal phenotypes; optionally wherein the neuronal phenotype comprises expressing one or more neuronal markers selected from DCX, TUJ1, NeuN, and MAP2; optionally the population of glioma cells exhibit the one or more neuronal phenotype in less than 14 days after the administering of the pharmaceutical composition.
- [Claim 86] The method of claim 83, wherein the population of glioma cells stop expressing one or more glial marker; optionally the one or more glial marker is selected from GFAP, Aldh1l1, S100 β and Sox9; optionally the population of glioma cells stop expressing the one or more glial

marker in less than 14 days after the administering of the pharmaceutical composition.

[Claim 87]

The method of claim 83, wherein the population of glioma cells transdifferentiate into neurons; optionally wherein the neurons are selected from glutamatergic neurons, GABAergic neurons, dopaminergic neurons; motor neurons, glycinergic neurons, serotonergic neurons, norepinephrinergic neurons, and sensory neurons; optionally wherein at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95% of the glioma cells in the population transdifferentiate into neurons.

[Claim 88]

The method of claim 83, wherein the population of glioma cells form one or more gliomas, and wherein the size of the glioma is reduced; optionally wherein the size of the glioma is reduced for at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95%.

[Claim 89]

A gene-of-interest (GOI) plasmid comprising an expression cassette comprising a transgene of interest and a pair of AAV ITR sequences flanking the expression cassette, wherein the AAV ITR located to the 3' end of the expression cassette does not have a functional terminal resolution site (trs) , and wherein the transgene encodes a NeuroD1 polypeptide, wherein the NeuroD1 polypeptide comprises an amino acid sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO: 1.

[Claim 90]

A host cell comprising the GOI plasmid of claim 89.

[Claim 91]

A method of producing a recombinant AAV comprising:

(a) culturing a host cell containing:

(i) an artificial genome comprising a cis expression cassette, wherein the cis expression cassette comprises a coding sequence encoding a NeuroD1 polypeptide, wherein the NeuroD1 polypeptide comprises an amino acid sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO: 1;

(ii) a trans expression cassette lacking AAV ITRs, wherein the trans expression cassette encodes an AAV Rep and Capsid proteins operably linked to expression control element that drive expression of the AAV Rep and capsid proteins in the host cell in culture, and supply the Rep and Capsid proteins in trans;

(iii) sufficient adenovirus helper functions to permit replication and packaging of the artificial genome by the AAV capsid proteins; and
(b) recovering the recombinant AAV encapsidating the artificial genome from the cell culture.

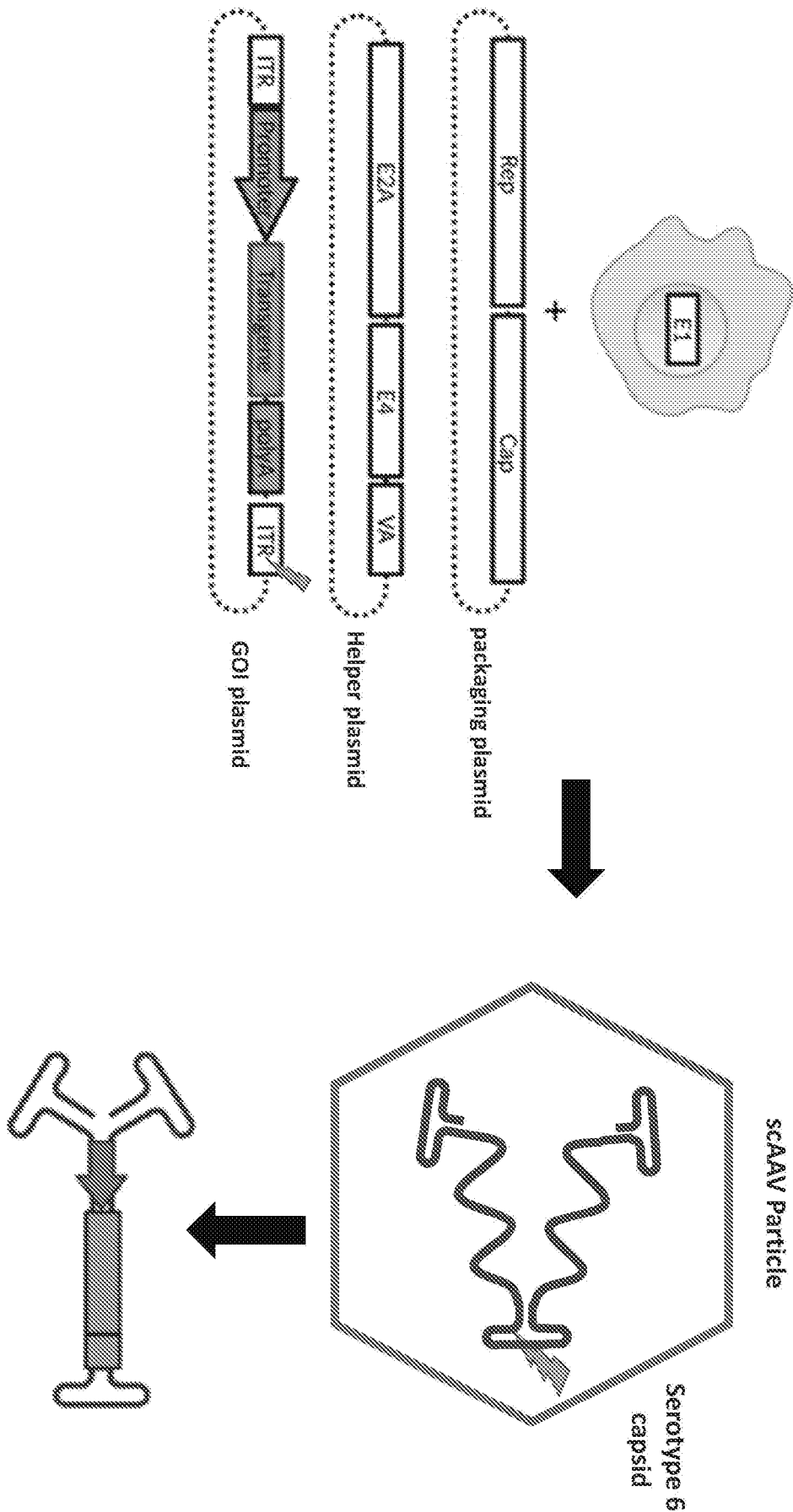
[Claim 92]

The method of claim 91, wherein the artificial genome is synthesized by the host cell using a GOI plasmid sequence as a replication template, wherein the GOI plasmid comprises the cis expression cassette flanked by a pair of AAV ITR sequences, wherein the AAV ITR located 3' to the cis expression cassette does not have a functional terminal resolution site (trs) .

[Claim 93]

A host cell comprising an artificial genome comprising the single-stranded self-complementary nucleic acid molecule of any one of claims 1 to 20.

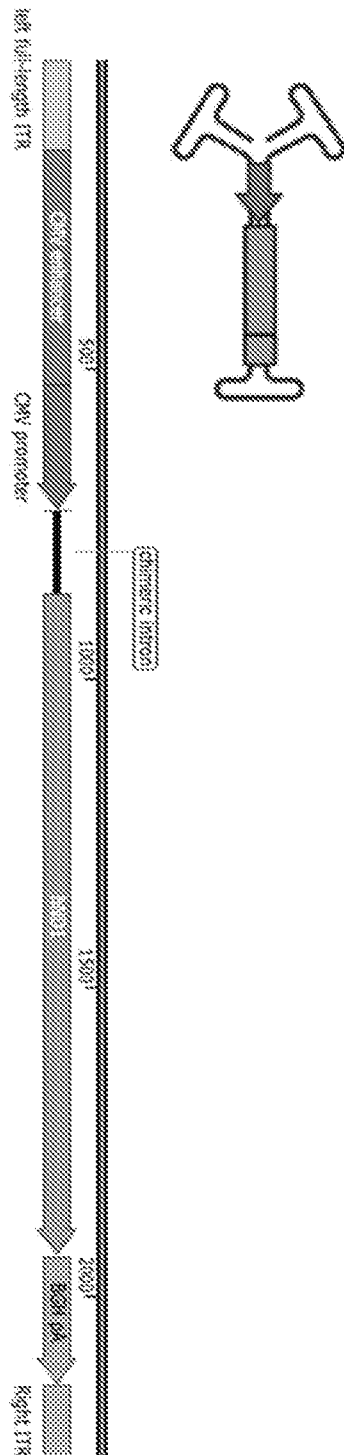
[Fig. 1]



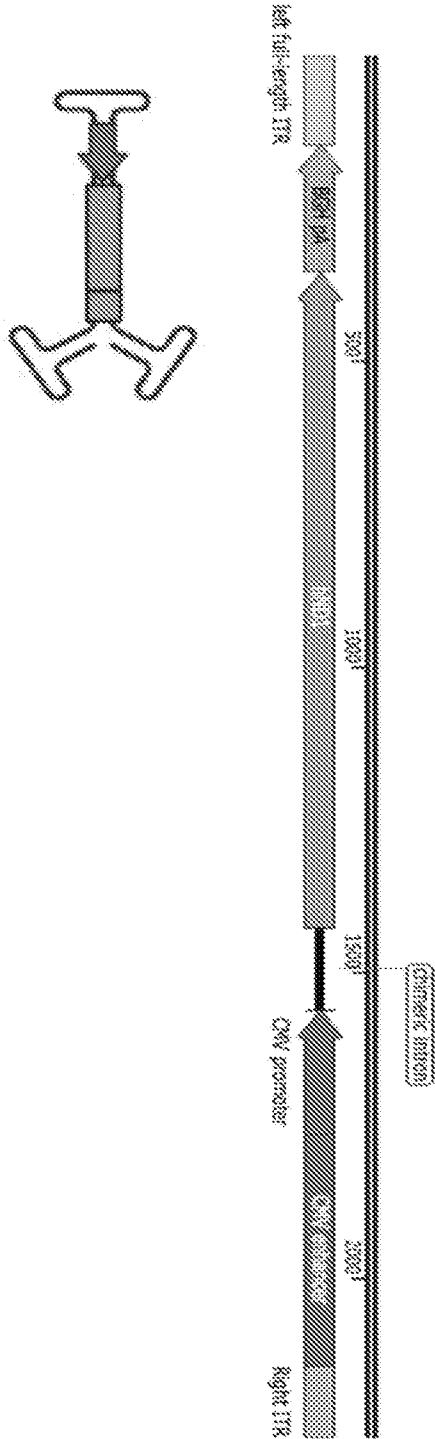
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[Fig. 2A]

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[Fig. 2B]



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[Fig. 2C]

<i>Mus musculus</i>	1	MTKSYSESGLMGEPPQGPSPSWTDECLSSQDEEHEADKKEDLEANN--AE--EDSLR--NGGTEETDE-DLEEE-----70
<i>Danio rerio</i>	1	MTKSYSEESMRLSSSS--NMTDKCHSSQDEROVDTSEPM--LM--DM--EDD----DMAQLNRLDE-DDEEE-----65
<i>Homo sapiens</i>	1	MTKSYSESGLMGEPPQGPSPSWTDECLSSQDEEHEADKKEDLETPM--AE--EDSLR--NGGTEETDE-DLEEE-----70
<i>Rattus norvegicus</i>	1	MTKSYSESGLMGEPPQGPSPSWTDECLSSQDEEHEADKKEDLEANN--AE--EDSLR--NGGTEETDE-DLEEE-----70
<i>Gallus gallus</i>	1	MTKSYSESGPAGLPPQGPSPSWMAAGLSPPAKPELEADKKEDLEALIGAE--EDALR--NGGTEETDELDDEEE---73
<i>Bos taurus</i>	1	MTKSYSESGLMGEPPQGPSPSWTDECLSSQDEEHEADKKEDLEANN--AE--EDSLR--NGGTEETDE-DLEEE-----70
<i>Mesocricetus auratus</i>	1	MTKSYSESGLMGEPPQGPSPSWTDECLSSQDEEHEADKKEDLEANN--AE--EDSLR--NGGTEETDE-DLEEE-----70
<i>Sus scrofa</i>	1	MTKSYSESGLMGEPPQGPSPSWTDECLSSQDEEHEADKKEDLEANN--AE--EDSLR--NGGTEETDE-DLEEE-----70
<i>Xenopus tropicalis</i>	1	MTKSYRENGLIQAETP--GSRGADDECLSQDE--HDLKKEGLDSLM--KEGDEDSLNNNGETNEEEDVMDDEEddd76
<i>Canis lupus familiaris</i>	1	MTKSYSESGLMGEPPQGPSPSWTDECLSPQDEEHEADKKEDLEANN--AE--EDSLR--NGGTEETDE-DLEEE-----70
<i>Ovis aries</i>	1	MTKSYSESGLMGEPPQGPSPSWTDECLSSQDEEHEADKKEDLEANN--AE--EDSLR--NGGTEETDE-DLEEE-----70
<i>Pan troglodytes</i>	1	MTKSYSESGLMGEPPQGPSPSWTDECLSSQDEEHEADKKEDLEANN--AE--EDSLR--NGGTEETDE-DLEEE-----70

[Fig. 2C CONTINUED]

<i>Mus musculus</i>	71	- EEEEEEDXXPKRRGPKKKKKTKAALERFLRRKKANARERRRRGGLNAALDMLKKVVPQYSKTQKLSKIEFLRLAKNY	149
<i>Danio rerio</i>	66	- EEEEDGDDTKPKRRGPKKKKKTKAARQKFKRRKKANARERRRRGGLNDALFSLRKVVPQYSKTQKLSKIEFLRLAKNY	144
<i>Homo sapiens</i>	71	- EEEETDXXPKRRGPKKKKKTKAALERFLRRKKANARERRRRGGLMAALDMLKKVVPQYSKTQKLSKIEFLRLAKNY	149
<i>Rattus norvegicus</i>	71	- EEEEEEDXXPKRRGPKKKKKTKAALERFLRRKKANARERRRRGGLMAALDMLKKVVPQYSKTQKLSKIEFLRLAKNY	149
<i>Gallus gallus</i>	74	- EEEEEEDTKQPKRRGPKKKKKTKAALERFLRRKKANARERRRRGGLMAALDMLKKVVPQYSKTQKLSKIEFLRLAKNY	152
<i>Bos taurus</i>	71	- EEEEEEDXXPKRRGPKKKKKTKAALERFLRRKKANARERRRRGGLMAALDMLKKVVPQYSKTQKLSKIEFLRLAKNY	149
<i>Mesocricetus auratus</i>	71	- EEEEEEDXXPKRRGPKKKKKTKAALERFLRRKKANARERRRRGGLMAALDMLKKVVPQYSKTQKLSKIEFLRLAKNY	148
<i>Sus scrofa</i>	71	- EEEEEEDTKQPKRRGPKKKKKTKAALERFLRRKKANARERRRRGGLMAALDMLKKVVPQYSKTQKLSKIEFLRLAKNY	149
<i>Xenopus tropicalis</i>	77	- EEEEEEDXXPKRRGPKKKKKTKAARVERFKVRRKKANARERRRRGGLMAALDMLKKVVPQYSKTQKLSKIEFLRLAKNY	156
<i>Canis lupus familiaris</i>	71	- EEEEEEDXXPKRRGPKKKKKTKAALERFLRRKKANARERRRRGGLMAALDMLKKVVPQYSKTQKLSKIEFLRLAKNY	149
<i>Ovis aries</i>	71	- EEEEEEDTKQPKRRGPKKKKKTKAALERFLRRKKANARERRRRGGLMAALDMLKKVVPQYSKTQKLSKIEFLRLAKNY	149
<i>Pan troglodytes</i>	71	- EEEEEEDXXPKRRGPKKKKKTKAALERFLRRKKANARERRRRGGLMAALDMLKKVVPQYSKTQKLSKIEFLRLAKNY	149

[Fig. 2C CONTINUED]

<i>Mus musculus</i>	150	IMALSELRSCKSPDLVSFVQTLCKGLSQPTTNLVAGCLQLNPTFTLPEQNMMPNLPASASTPAPPSYQSPGLPSP	229
<i>Danio rerio</i>	145	IMALSETLRSCKSPDLMSFVQALCKGLSQPTTNLVAGCLQLNPTFTLPEQNMMPNQLASASTSALPYSYQTPGLPSP	224
<i>Homo sapiens</i>	150	IMALSETLRSCKSPDLVSFVQTLCKGLSQPTTNLVAGCLQLNPTFTLPEQNMMPNLPASASTPAPPSYQSPGLPSP	229
<i>Rattus norvegicus</i>	150	IMALSETLRSCKSPDLVSFVQTLCKGLSQPTTNLVAGCLQLNPTFTLPEQNMMPNLPASASTPAPPSYQSPGLPSP	229
<i>Gallus gallus</i>	153	IMALSELRSCKSPDLVSFVQTLCKGLSQPTTNLVAGCLQLNPTFTLPEQNMMPNLPAGAPYAPPPYASPGLPSP	232
<i>Bos taurus</i>	150	IMALSELRSCKSPDLVSFVQTLCKGLSQPTTNLVAGCLQLNPTFTLPEQNMMPNLPASASTPAPPSYQSPGLPSP	229
<i>Mesocricetus auratus</i>	149	IMALSETLRSCKSPDLVSFVQTLCKGLSQPTTNLVAGCLQLNPTFTLPEQNMMPNLPASASTPAPPSYQSPGLPSP	228
<i>Sus scrofa</i>	150	IMALSELRSCKSPDLVSFVQTLCKGLSQPTTNLVAGCLQLNPTFTLPEQNMMPNLPASASTPAPPSYQSPGLPSP	229
<i>Xenopus tropicalis</i>	157	IMALSELRSCKSPDLVSFVQTLCKGLSQPTTNLVAGCLQLNPTFTLPEQNMMPNLPASASTPAPPSYQSPGLPSP	236
<i>Canis lupus familiaris</i>	150	IMALSETLRSCKSPDLVSFVQTLCKGLSQPTTNLVAGCLQLNPTFTLPEQNMMPNLPACAPPPAPPPYQSPGLPSP	229
<i>Ovis aries</i>	150	IMALSELRSCKSPDLVSFVQTLCKGLSQPTTNLVAGCLQLNPTFTLPEQNMMPNLPASASTPAPPSYQSPGLPSP	229
<i>Pan troglodytes</i>	150	IMALSELRSCKSPDLVSFVQTLCKGLSQPTTNLVAGCLQLNPTFTLPEQNMMPNLPASASTPAPPSYQSPGLPSP	229

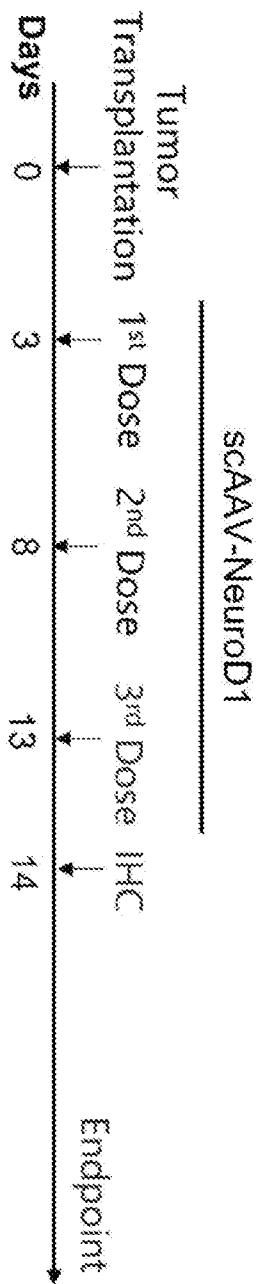
[Fig. 2C CONTINUED]

<i>Mus musculus</i>	230	PYGTMDSNRRFHKKRPPHAYSAALEPPFEESPLTDCTSPSFDGPLSPPLSINGMFSFKHEPSAEFEKNYAFTHRRPAATL-	308
<i>Danio rerio</i>	225	PYGTMDSSSHITHMRD---HAYGSALKEPFDITLTDCTSPSFDGPLSPPLSVNGMFSFKHEPSSEFEKNYAFTHRRQAAGL-	301
<i>Homo sapiens</i>	230	PYGTMDSNRRFHKKRPPHAYSAALEPPFEESPLTDCTSPSFDGPLSPPLSINGMFSFKHEPSAEFEKNYAFTHRRPAATL-	308
<i>Rattus norvegicus</i>	230	PYGTMDSNRRFHKKRPPHAYSAALEPPFEESPLTDCTSPSFDGPLSPPLSINGMFSFKHEPSIEFEKNYAFTHRRPAATL-	308
<i>Gallus gallus</i>	233	PYGTMDSNHLFHLKPP-HAYGSALEPPFEGLPEGADPTDGPLSPPLSINGMFSFKHEPSADPEDKSYAFTHRRPAATL-	310
<i>Bos taurus</i>	230	PYGTMDSNRRFHKKRPPHAYSAALEPPFEESPLTDCTSPSFDGPLSPPLSINGMFSFKHEPSAEFEKNYAFTHRRPAATL-	308
<i>Mesocricetus auratus</i>	229	PYGTMDSNRRFQKKRPPHAYSATLEPPFEESPLTDCTSPSFDGPLSPPLSINGMFSFKHEPSAEFEKNYAFTHRRPAATL-	307
<i>Sus scrofa</i>	230	PYGTMDSNRRFHKKRPPHAYSAALEPPFEESPLTDCTSPSFDGPLSPPLSINGMFSFKHEPSAEFEKNYAFTHRRPAATL-	308
<i>Xenopus tropicalis</i>	237	PYGTMDSNRRFHMRD--HGYGSALKEPFDISTVTDCTSPSFDGPLSPPLSVNGMFTTKHEPSAEYOKNYAFTHRRPAATMA	314
<i>Canis lupus familiaris</i>	230	PYGTMDSNRRFHKKRPPHAYSAALEPPFEESPLTDCAAPAFDGPLSPPLSINGMFSFKHEPSAEFEKSYAFTHRRPAATL-	308
<i>Ovis aries</i>	230	PYGTMDSNRRFHKKRPPHAYSAALEPPFEESPLTDCTSPSFDGPLSPPLSINGMFSFKHEPSAEFEKNYAFTHRRPAATL-	308
<i>Pan troglodytes</i>	230	PYGTMDSNRRFHKKRPPHAYSAALEPPFEESPLTDCTSPSFDGPLSPPLSINGMFSFKHEPSAEFEKNYAFTHRRPAATL-	308

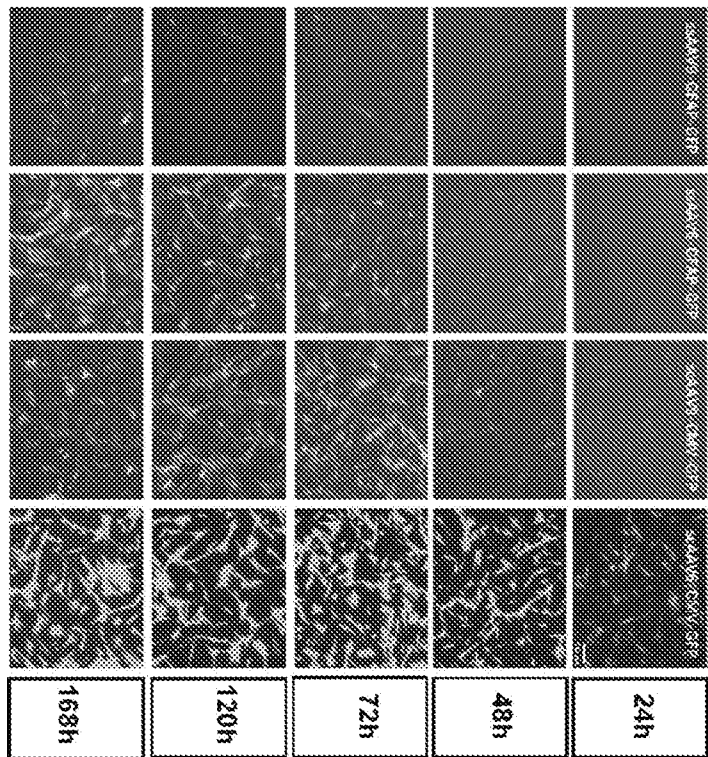
[Fig. 2C CONTINUED]

<i>Mus musculus</i>	309	AAQSHKSLFSSAAAPRCEIPIDNIPSDSHHERRMSAQLNALFHD-	357
<i>Danio rerio</i>	302	AAQSHVASL-VAGSTQCDIPMENIPSYDQSHHERRVMAQLNALFHD-	350
<i>Homo sapiens</i>	309	AAQSHKSLF-SGTAAPRCEIPIDNIPSDSHHERRMSAQLNALFHD-	356
<i>Rattus norvegicus</i>	309	AAQSHKSLFSSAAAPRCEIPIDNIPSDSHHERRMSAQLNALFHD-	357
<i>Gallus gallus</i>	311	PAAPAHAAVF-SGAA-RCELPADQLAPYEGPHHENVLSAQLSALFHE-	357
<i>Bos taurus</i>	309	AAQSHKSLF-SGASAPRCEIPIDNIPSDSHHERRMSAQLNALFHD-	356
<i>Mesocricetus auratus</i>	308	AAQSHKSLF-SGATAPRCEIPIDNIPSDSHHERRMSAQLNALFHD-	355
<i>Sus scrofa</i>	309	AAQSHKSLF-SGAAAPRCEIPIDNIPSDSHHERRMSAQLNALFHD-	356
<i>Xenopus tropicalis</i>	315	AAQSHKSLF-STGAPRCEIPIDNIPSDSHHERRMSAQLNALFHD-	361
<i>Canis lupus familiaris</i>	309	AAQSHASVF-SGAAPRCDIPIDSVVTFESHERRMSAQLNALFHD-	356
<i>Ovis aries</i>	309	AAQSHKSLF-SGAAPRCEIPIDNIPSDSHHERRMSAQLNALFHD-	356
<i>Pan troglodytes</i>	309	AAQSHKSLF-SGTAAPRCEIPIDNIPSDSHHERRMSAQLNALFHD-	356

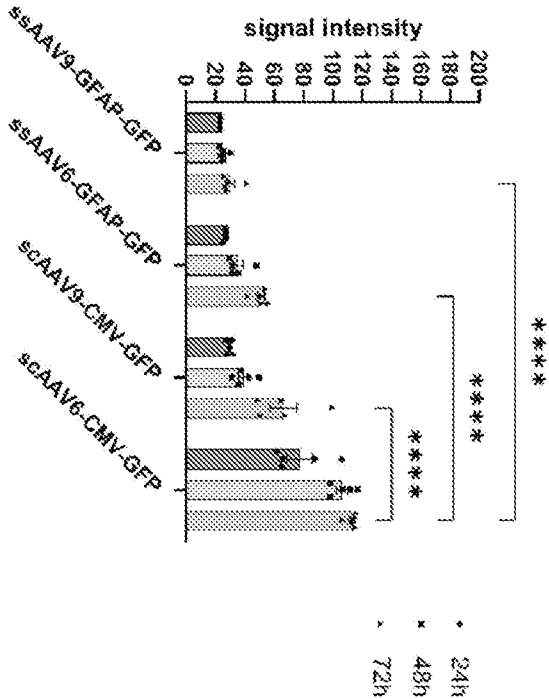
[Fig. 3]



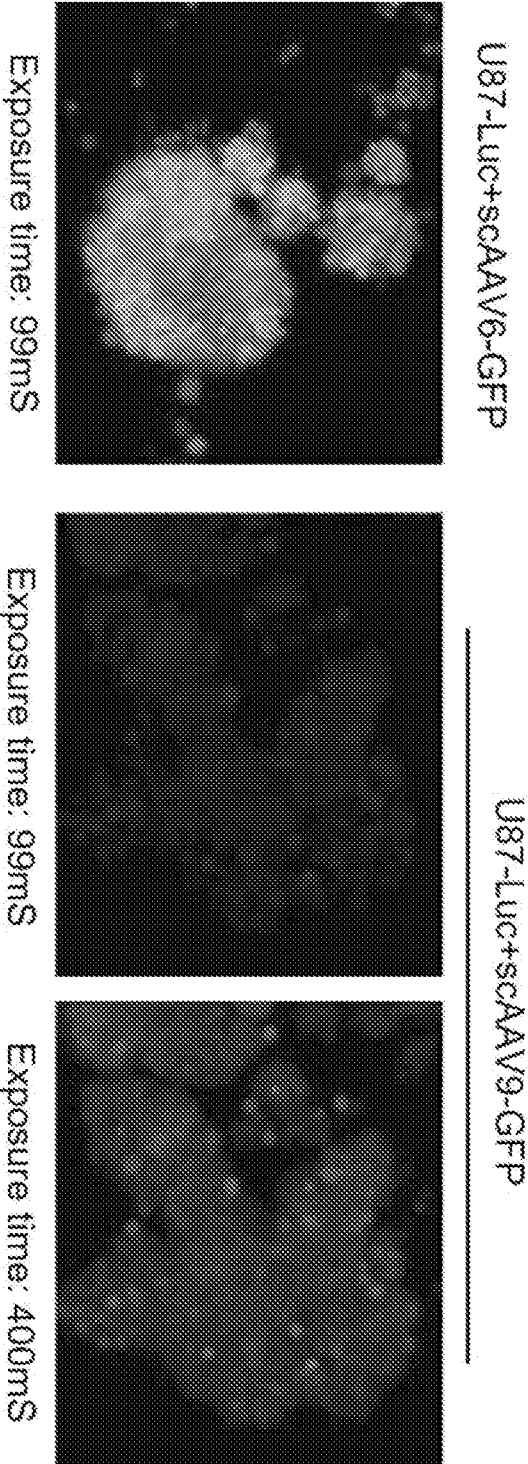
[Fig. 4A]



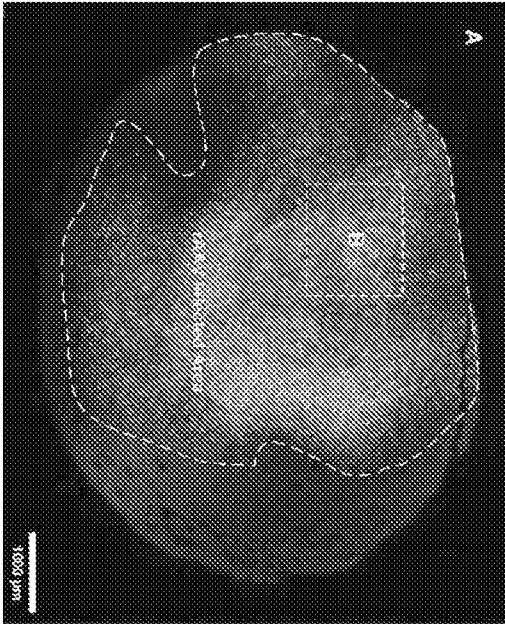
[Fig. 4B]



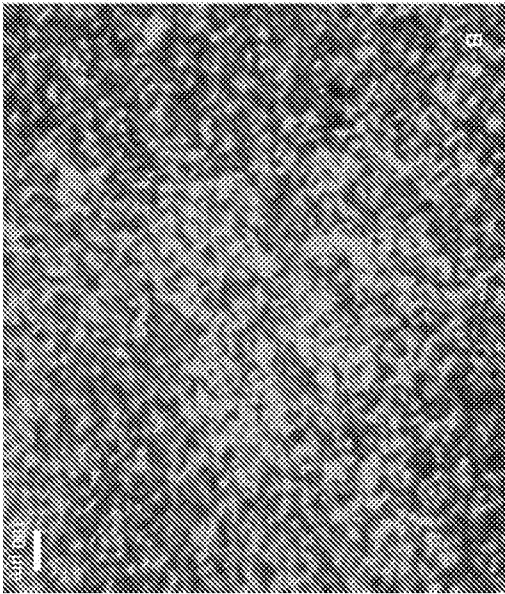
[Fig. 4C]



[Fig. 5A]



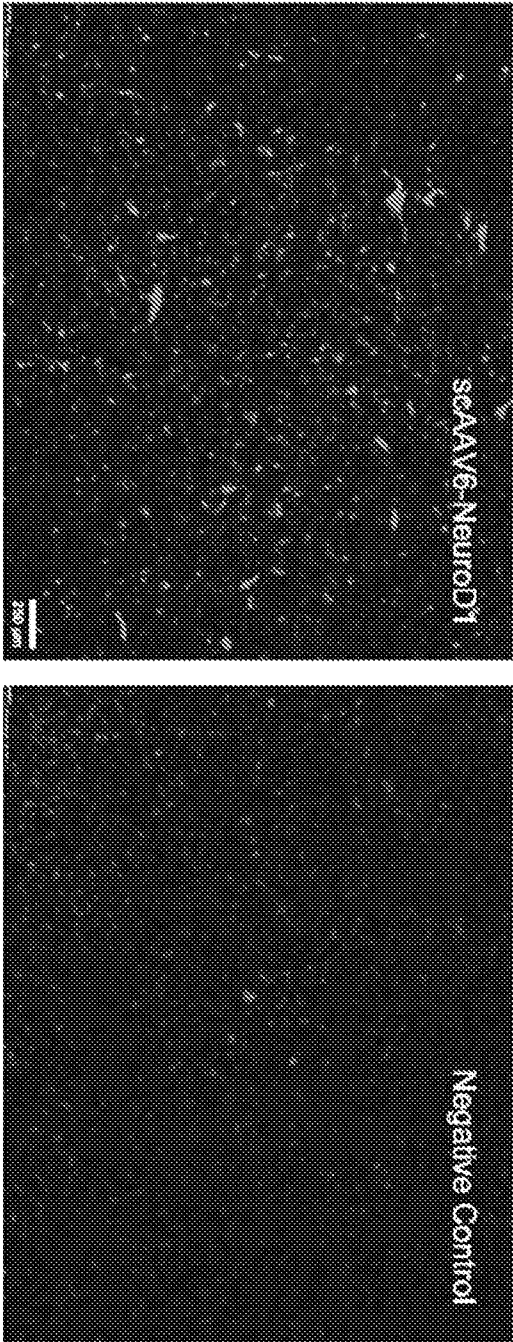
[Fig. 5B]



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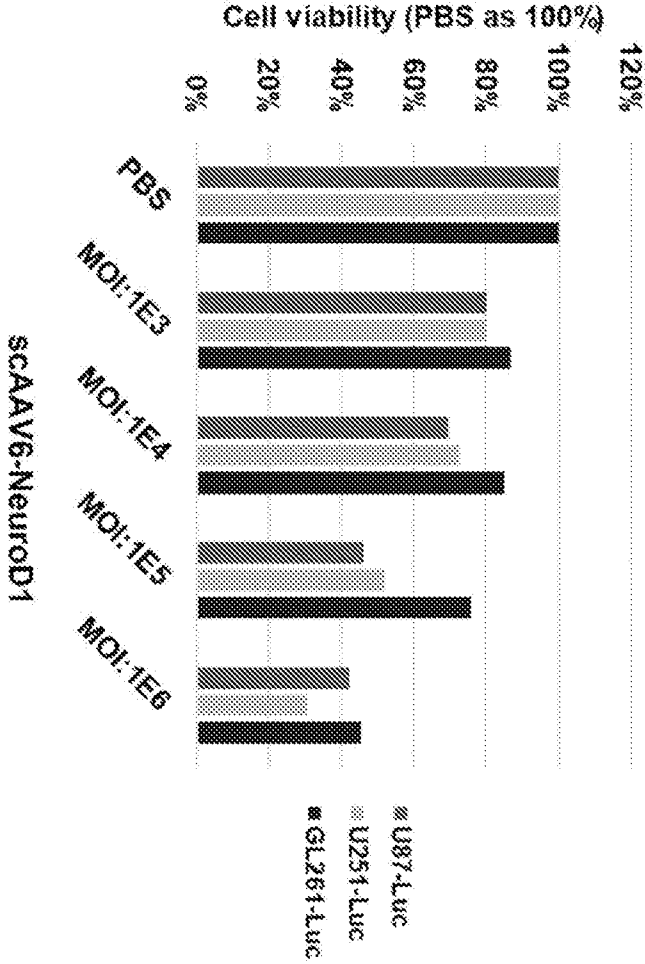
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[Fig. 6]

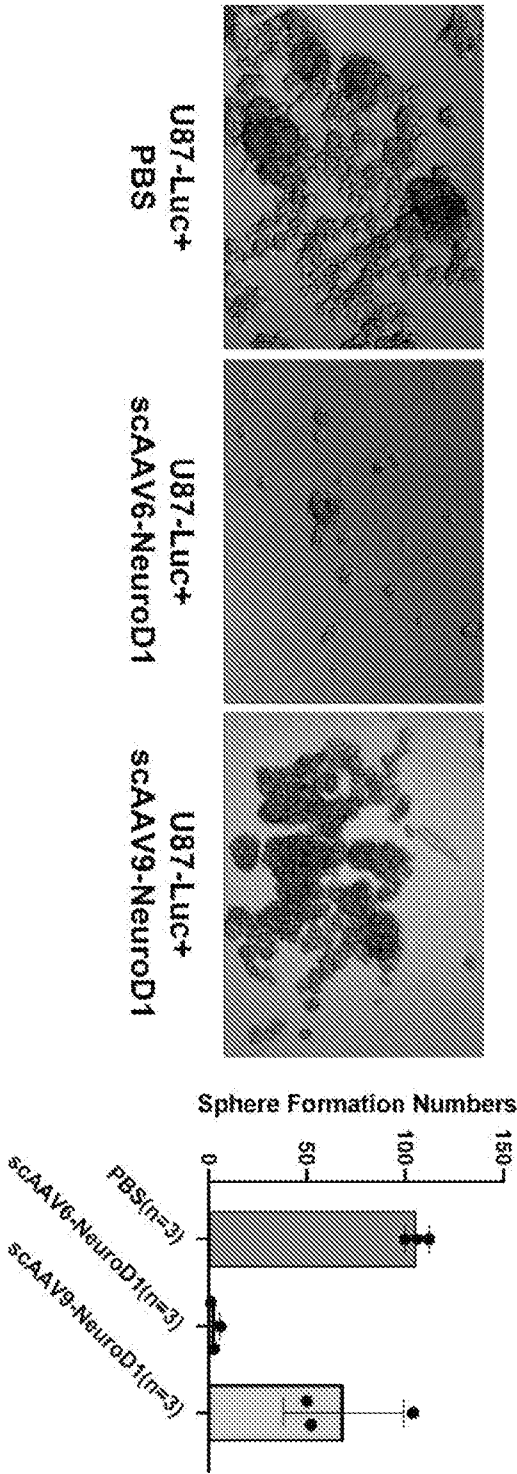


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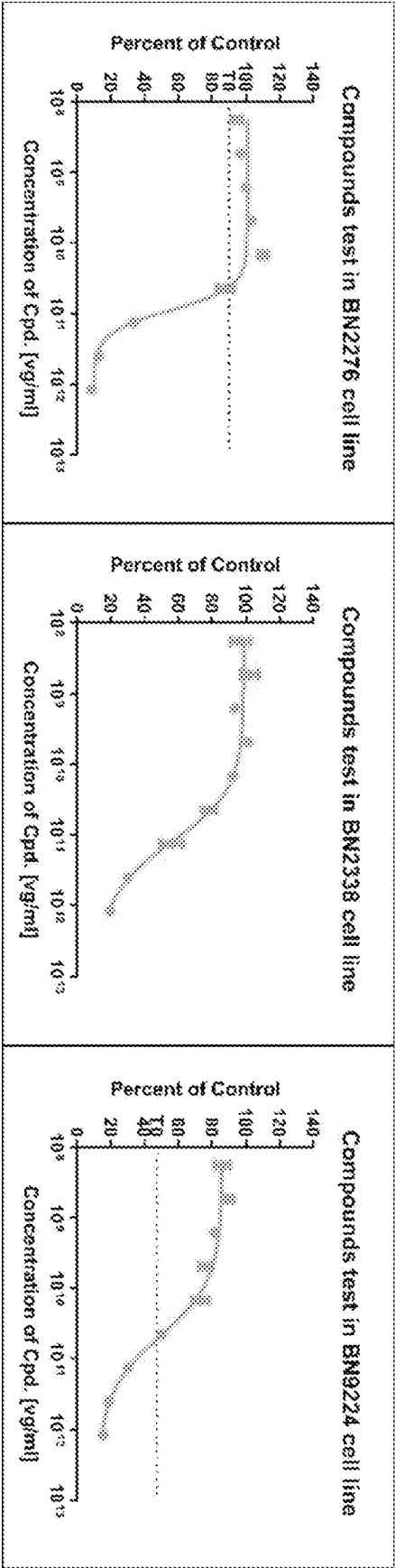
[Fig. 7]



[Fig. 8]

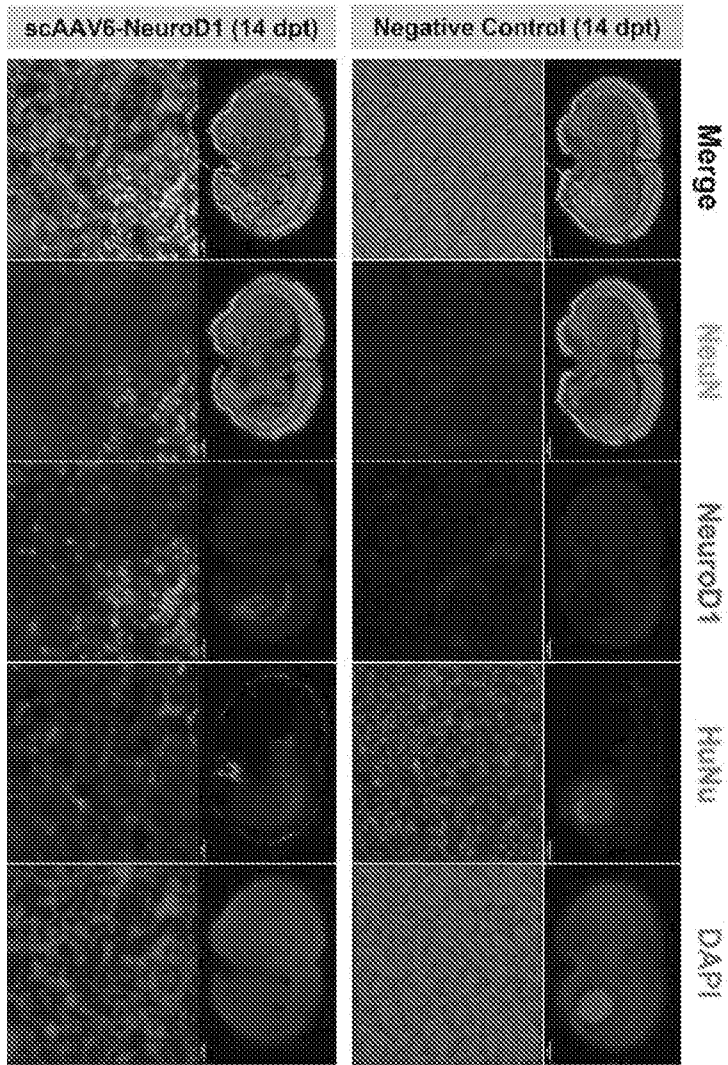


[Fig. 9]

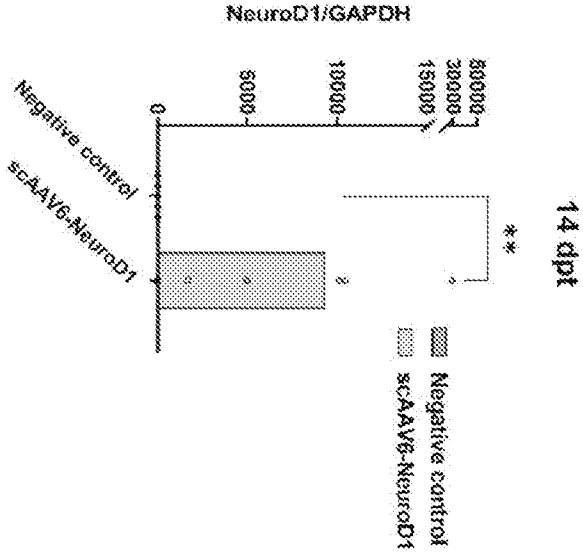


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[Fig. 10A]



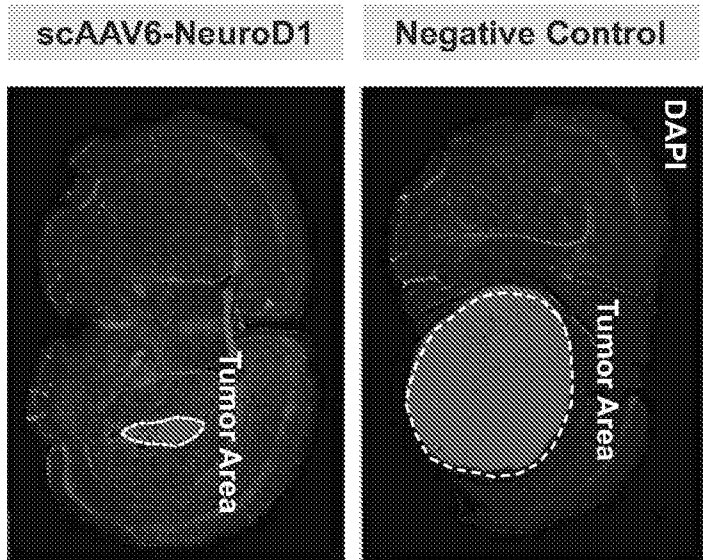
[Fig. 10B]



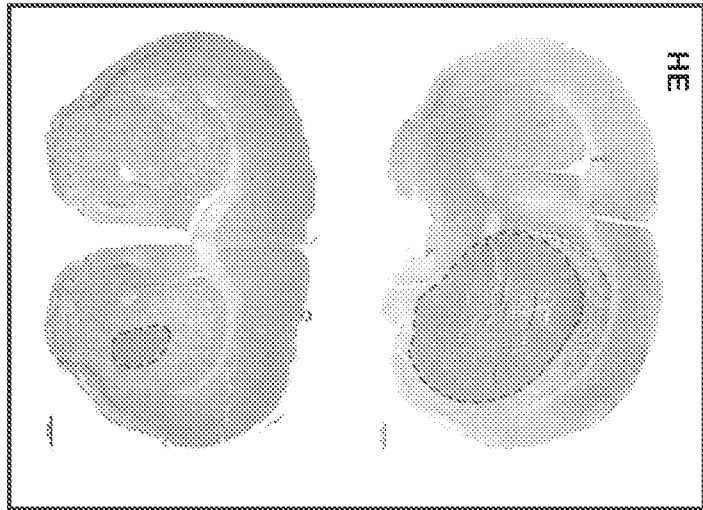
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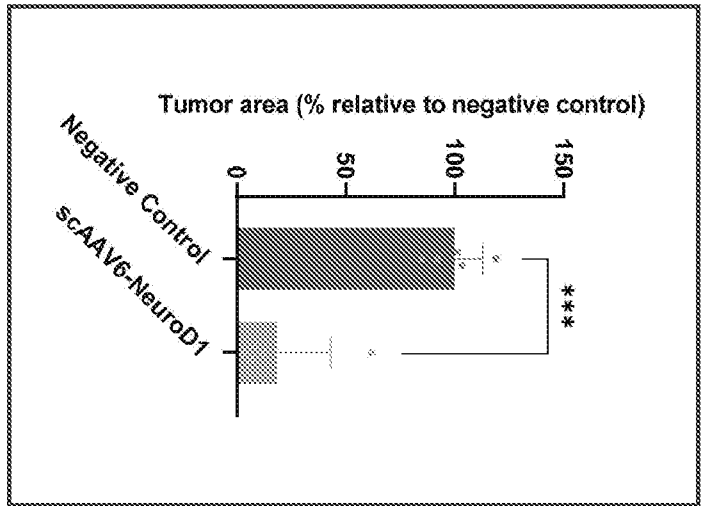
[Fig. 11A]



[Fig. 11B]



[Fig. 11C]

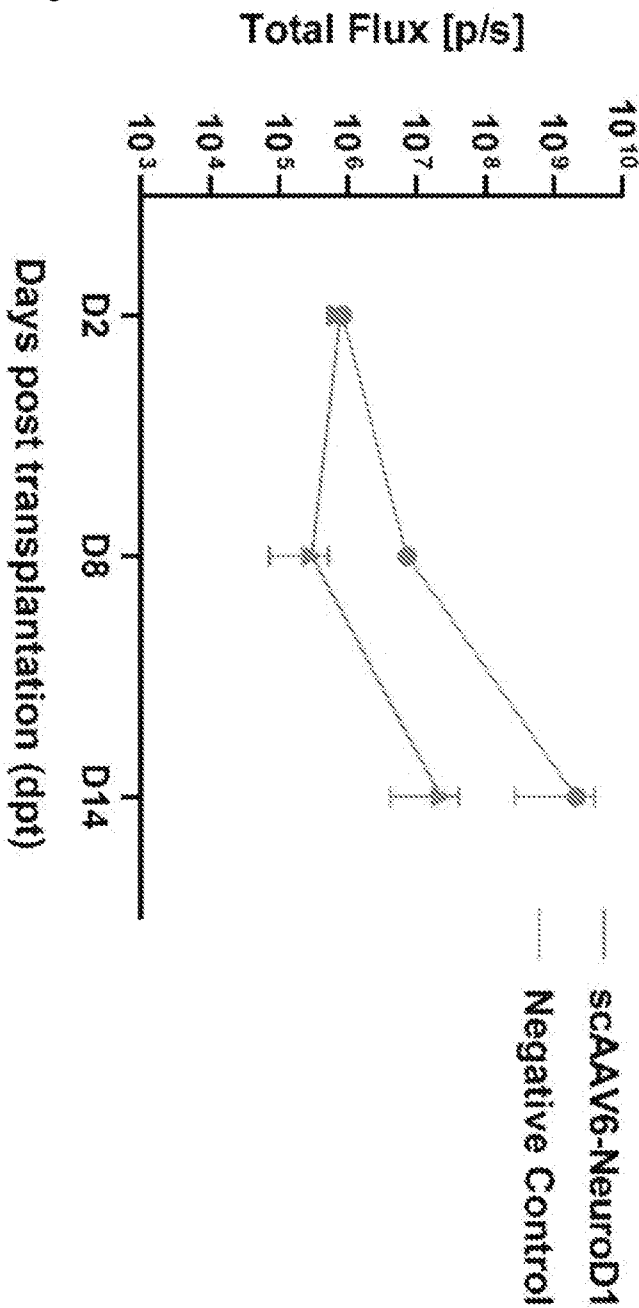


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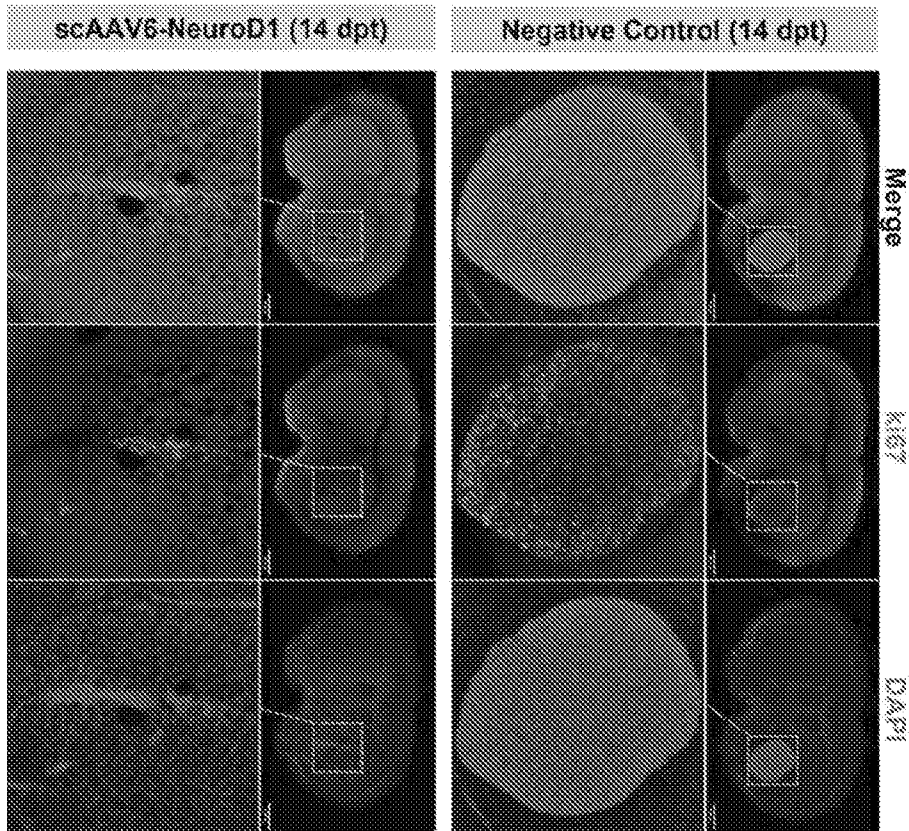
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[Fig. 12]

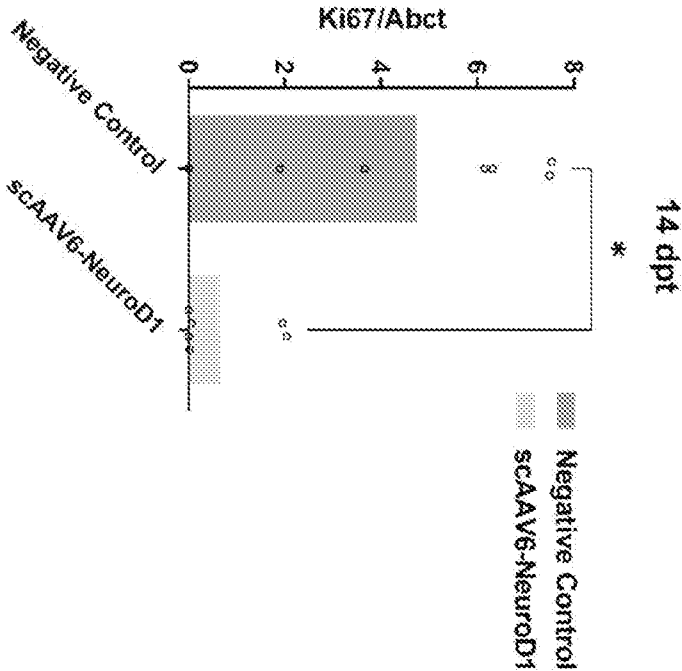


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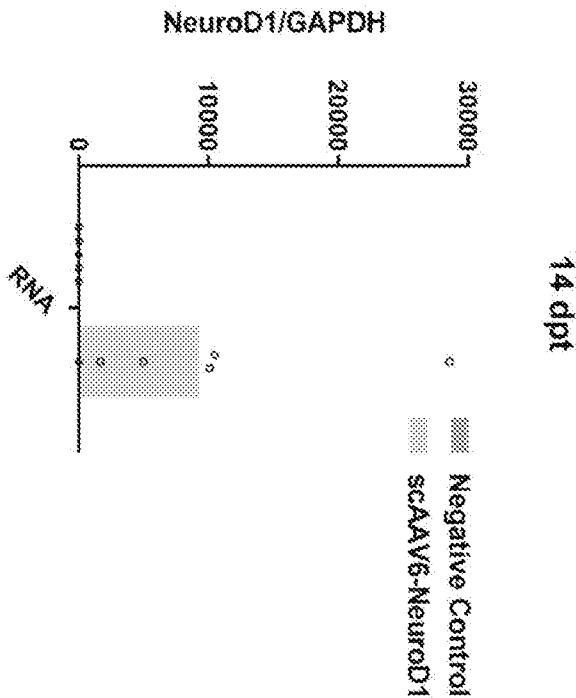
[Fig. 13]



[Fig. 14A]

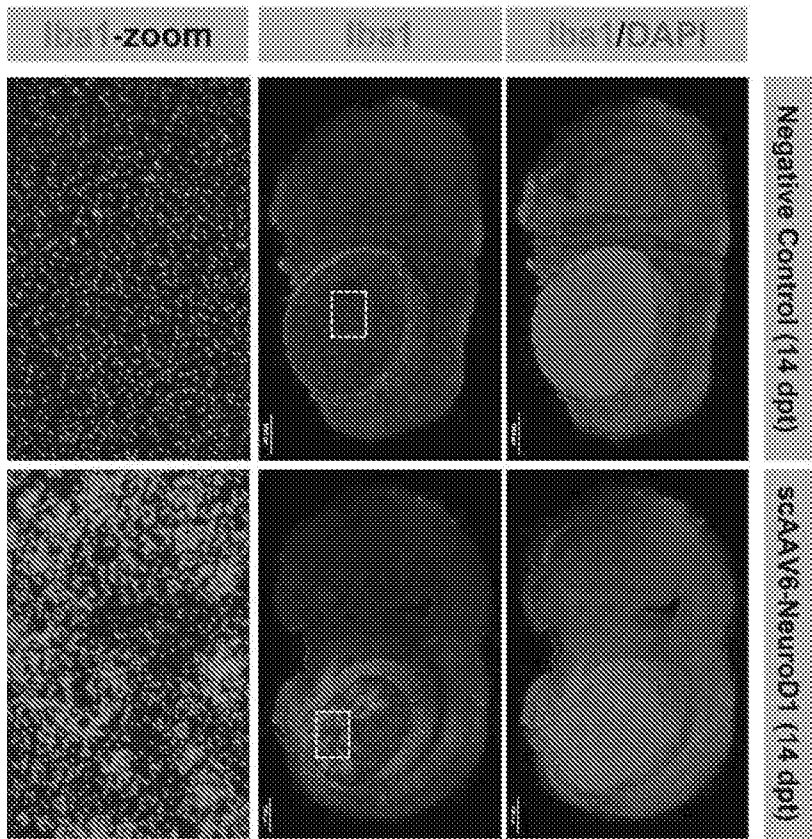


[Fig. 14B]



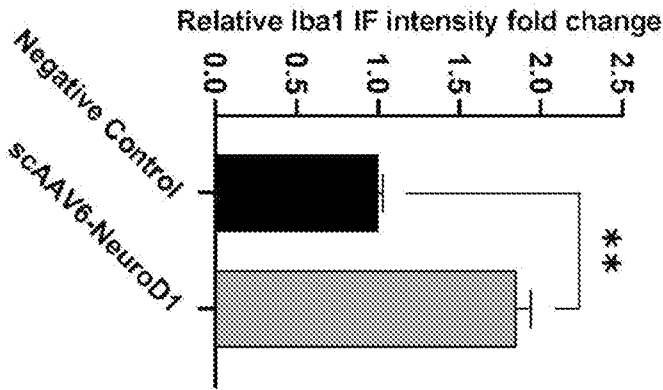
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[Fig. 15A]

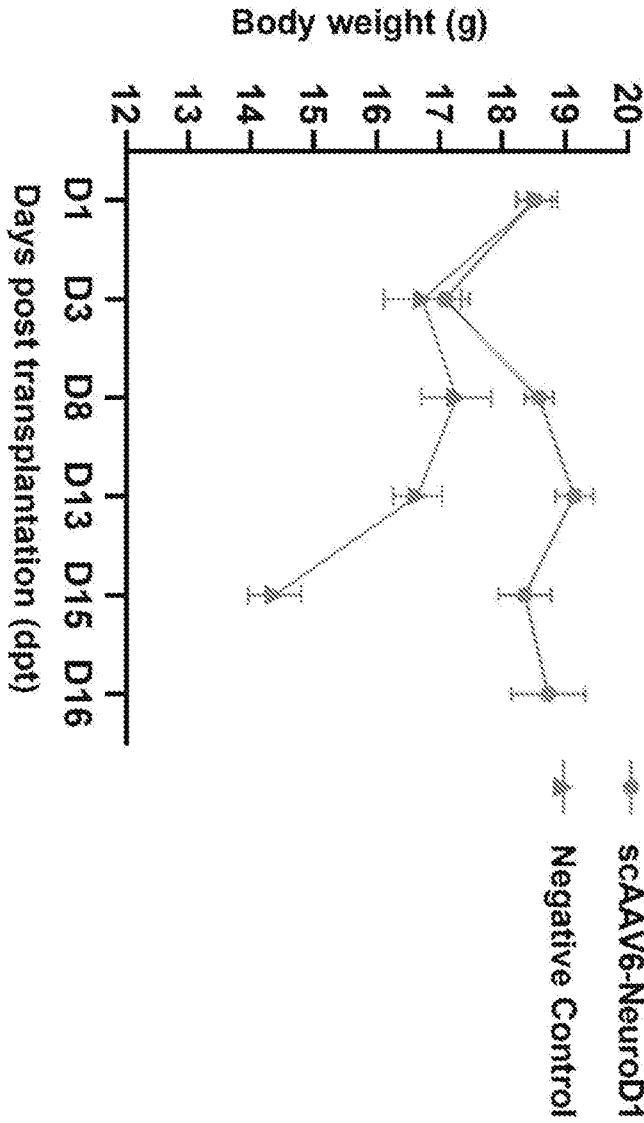


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[Fig. 15B]

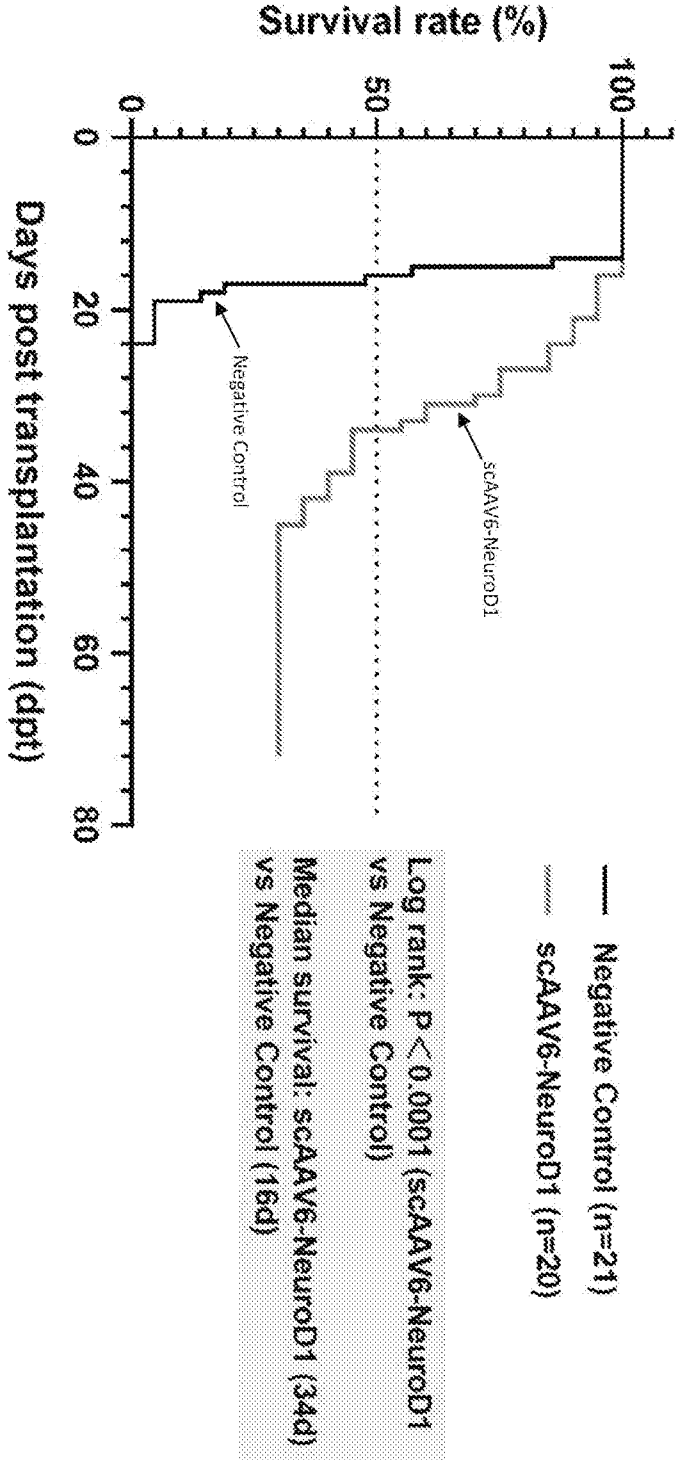


[Fig. 16]



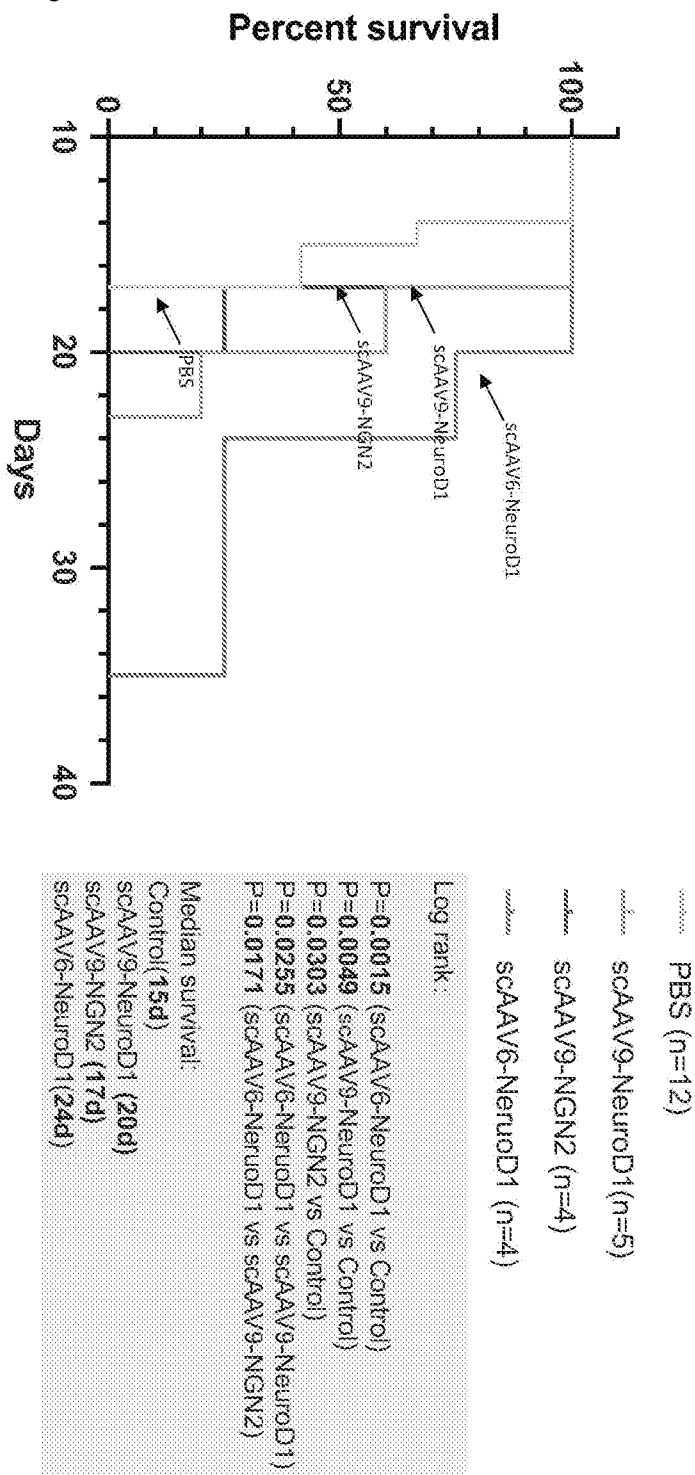
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[Fig. 17]



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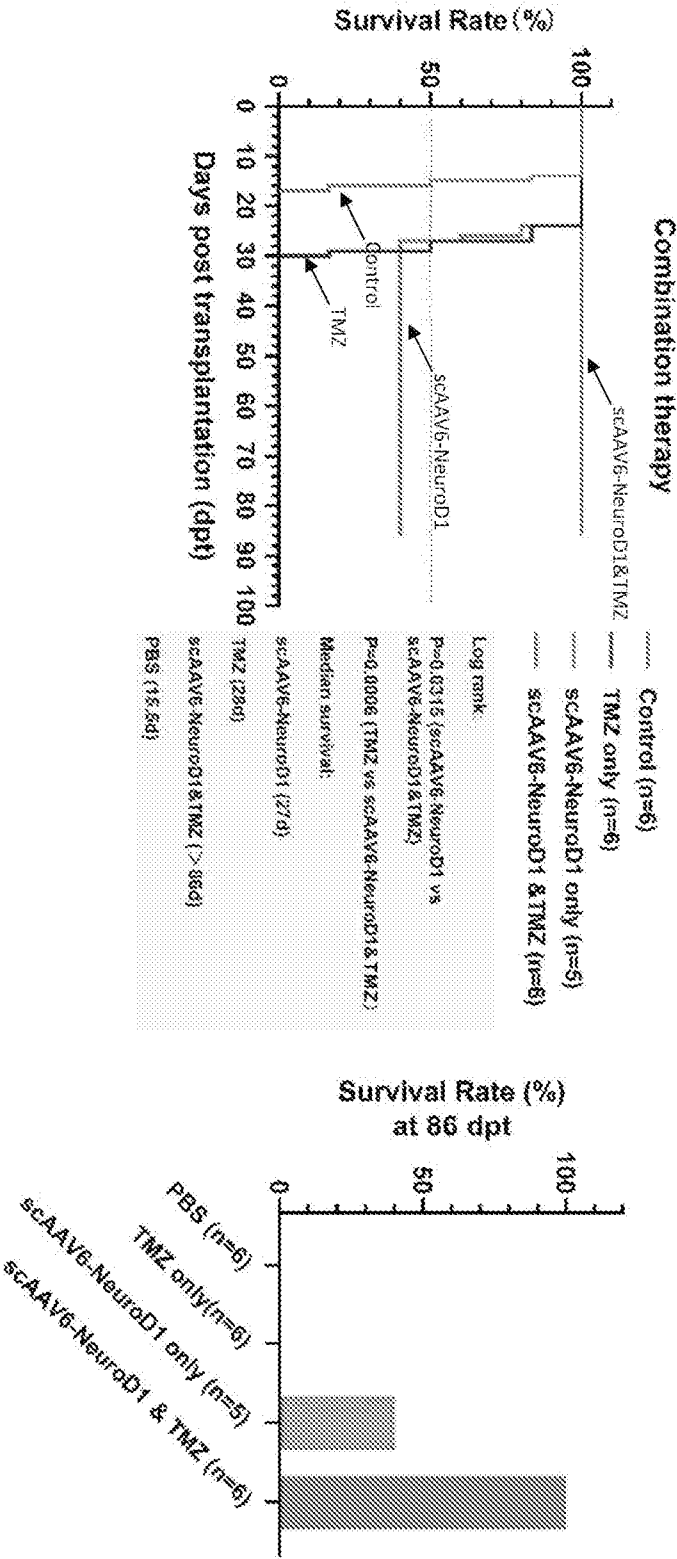
[Fig. 18]



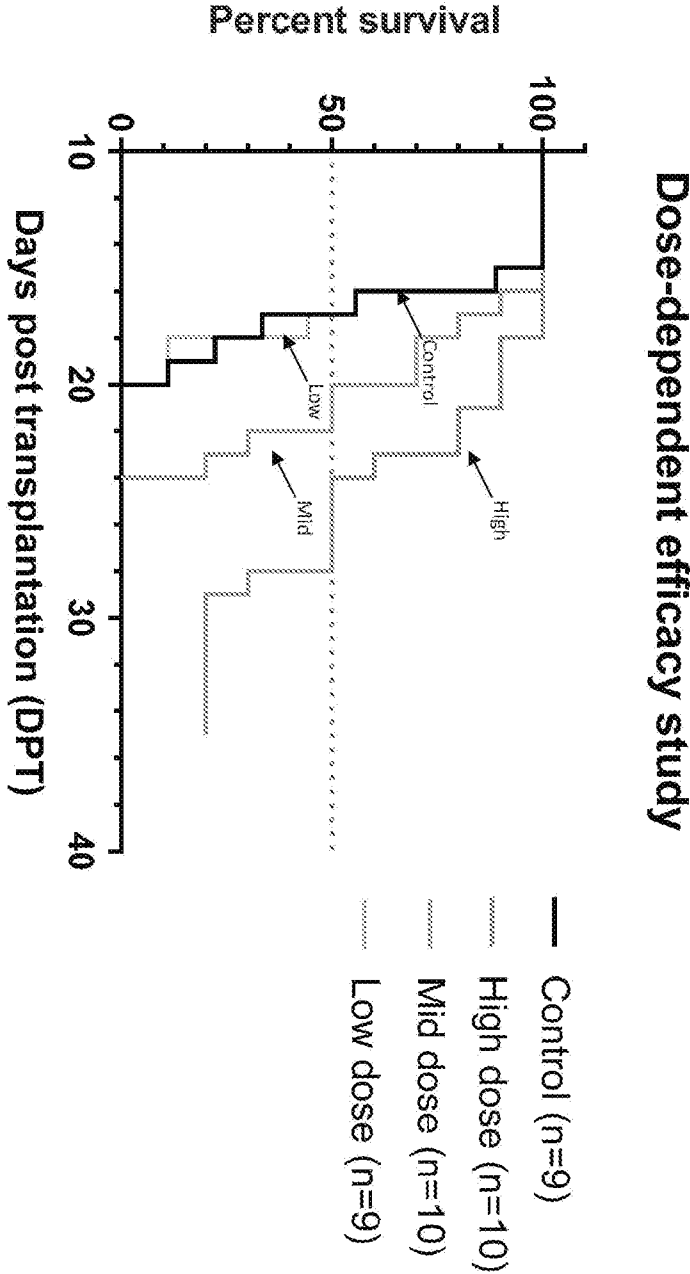
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[Fig. 19]

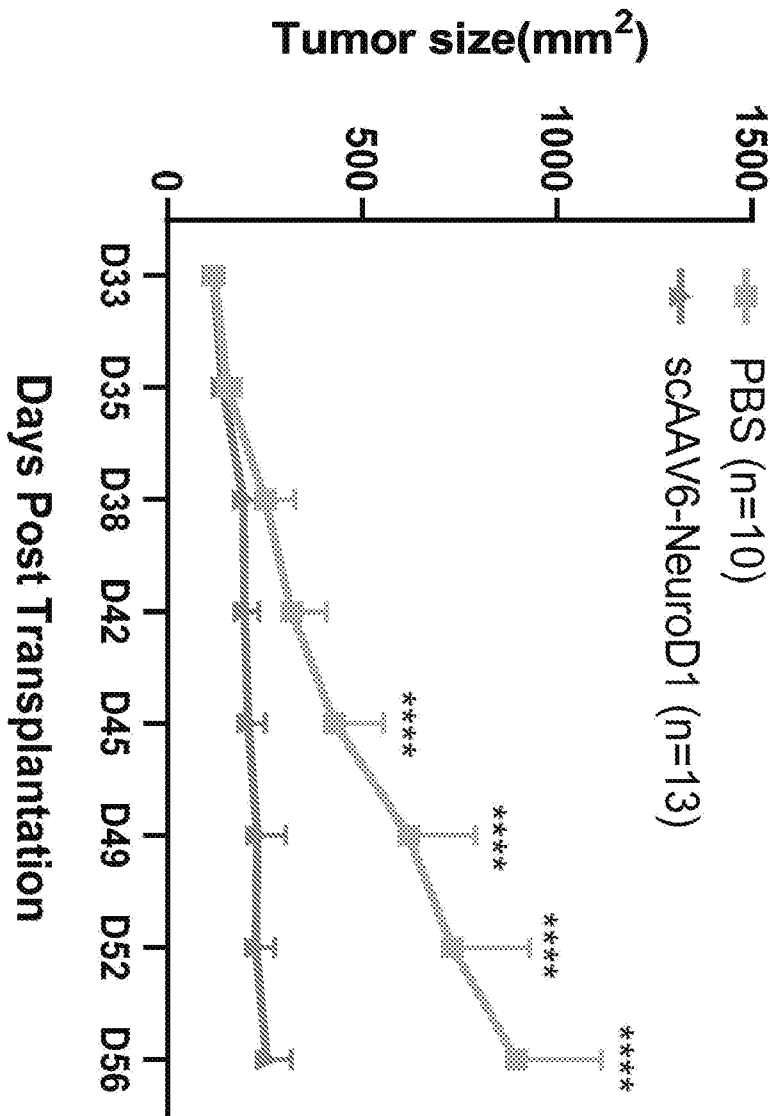
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[Fig. 20]

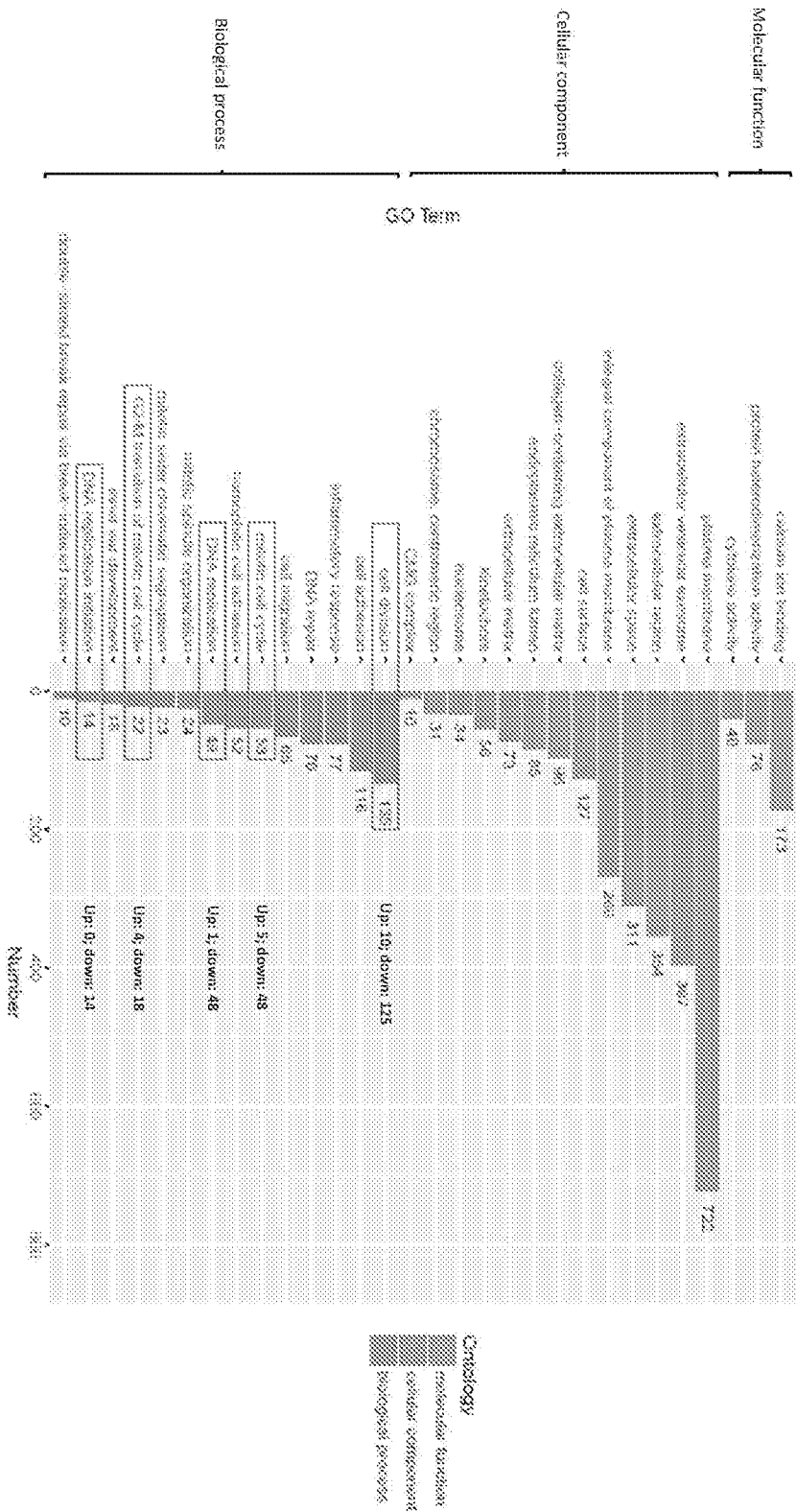


[Fig. 21]



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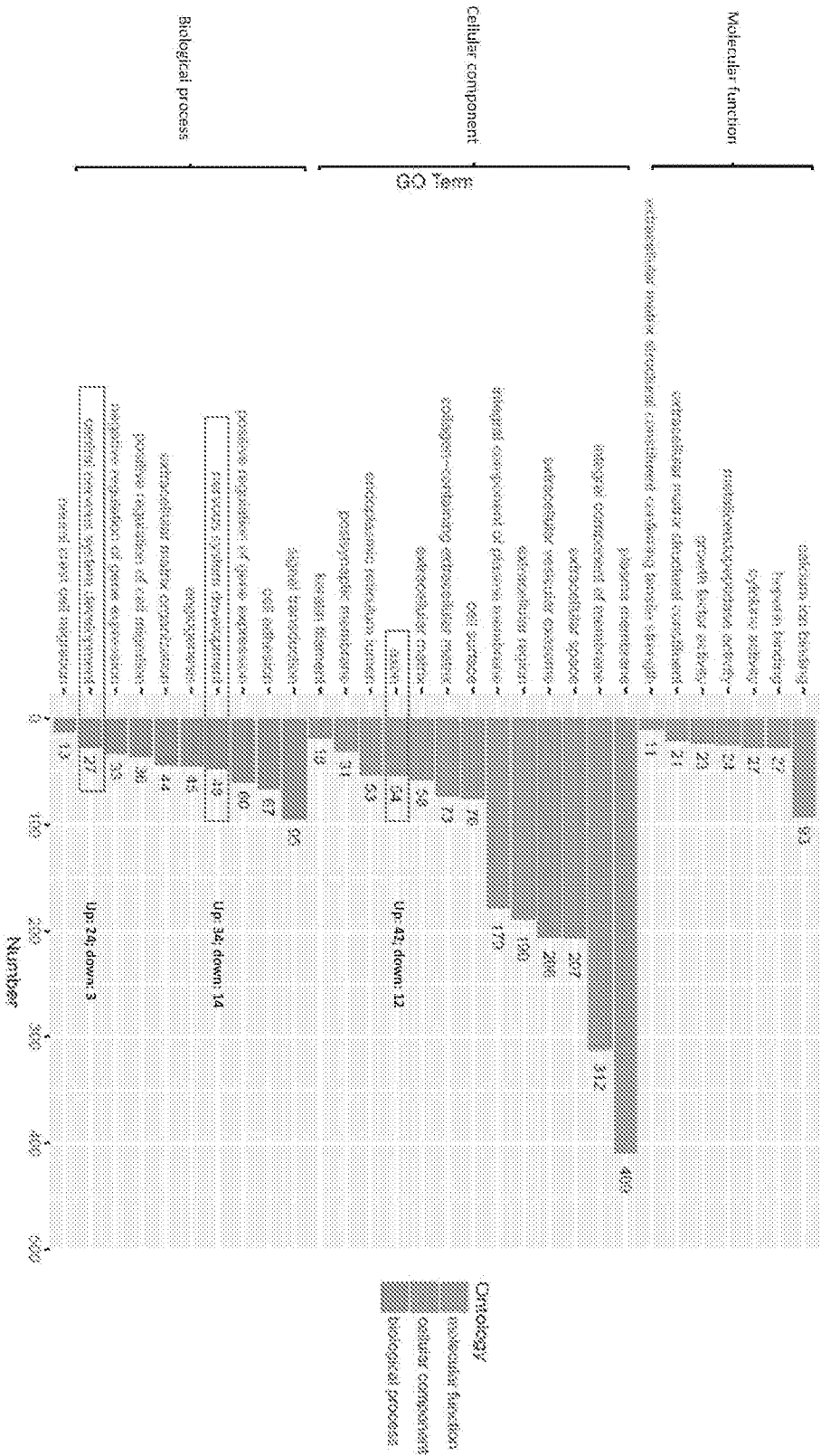
[Fig. 22]



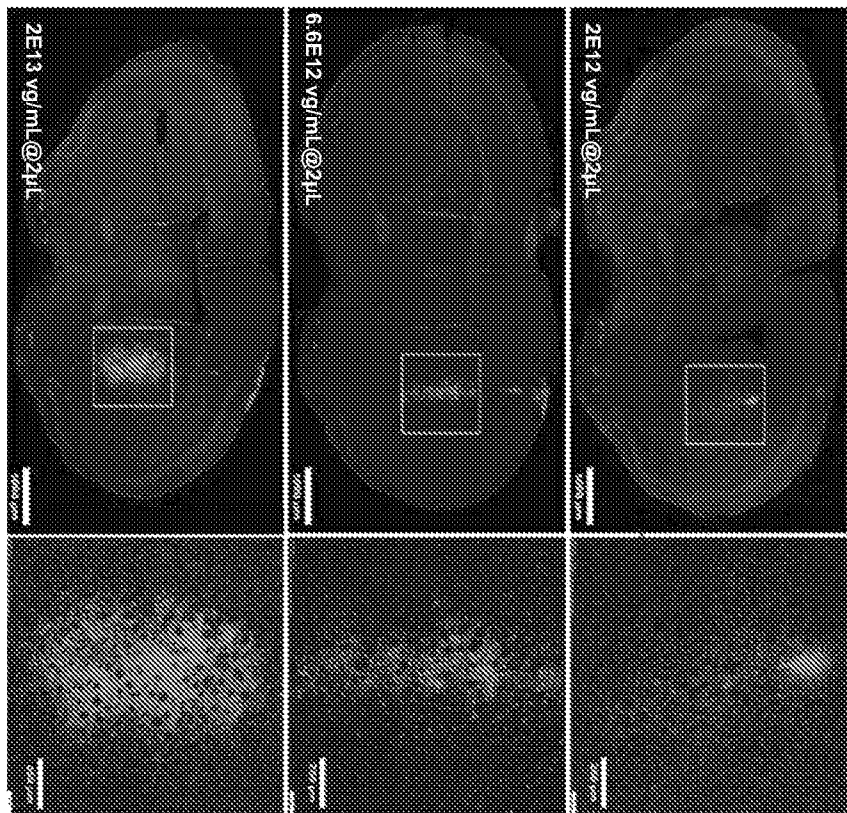
[Fig. 23]



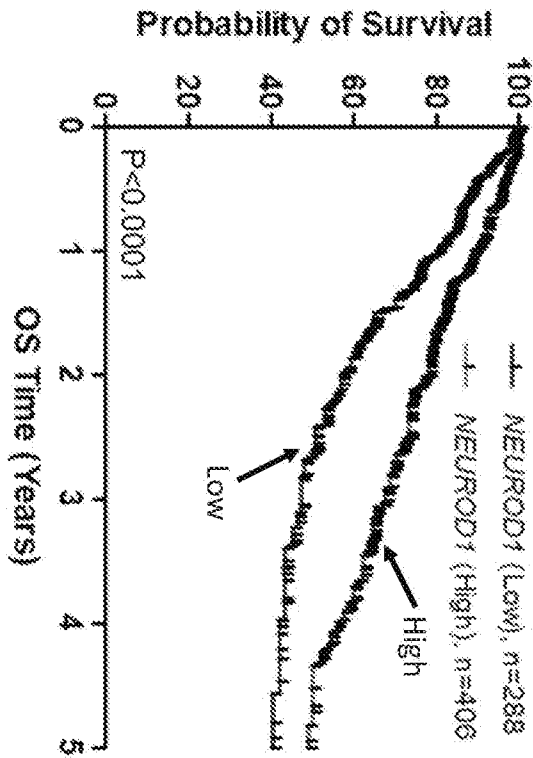
[Fig. 24]



[Fig. 25]



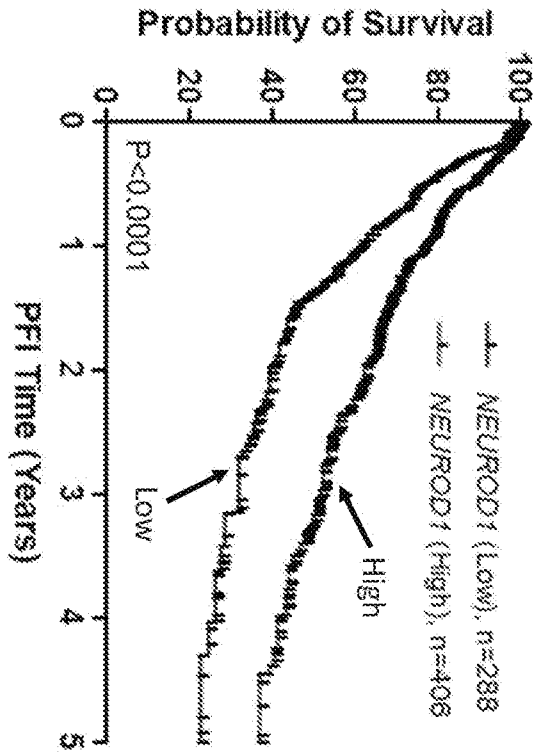
[Fig. 26A]



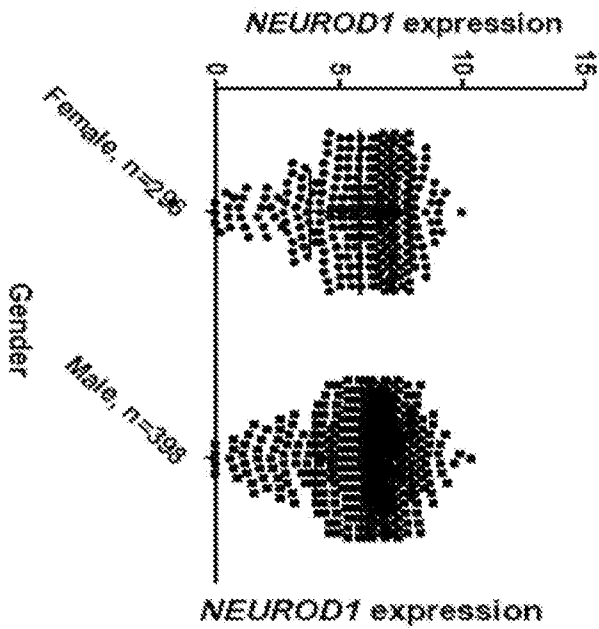
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[Fig. 26B]

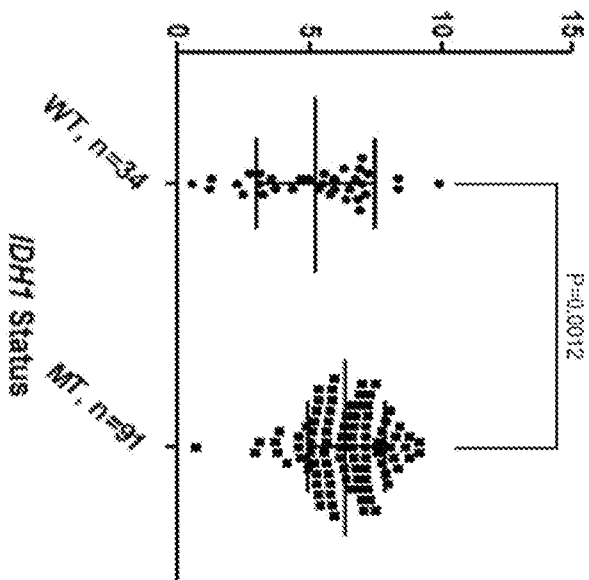


[Fig. 27A]

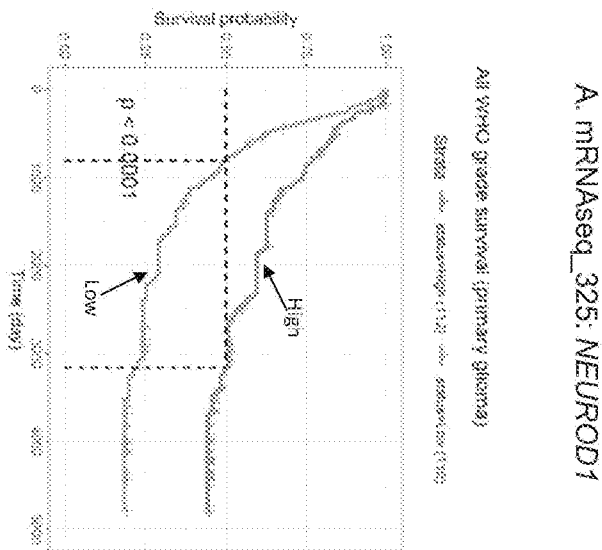


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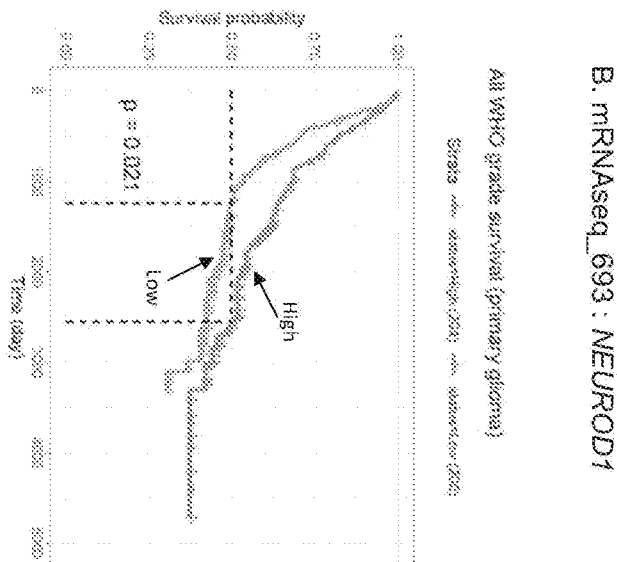
[Fig. 27B]



[Fig. 28A]



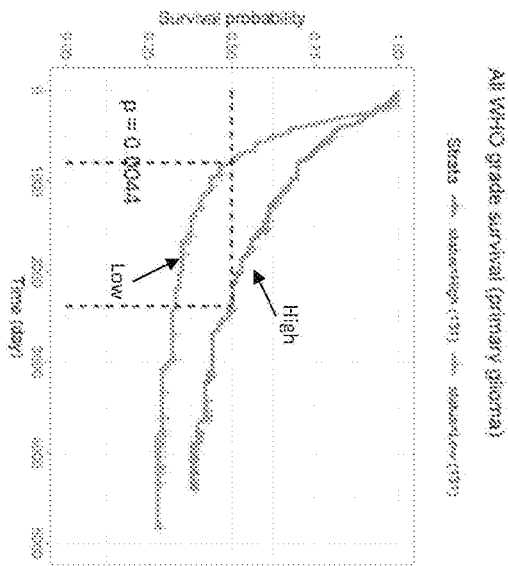
[Fig. 28B]



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[Fig. 28C]



C. mRNA-array_301 : NEUROD1