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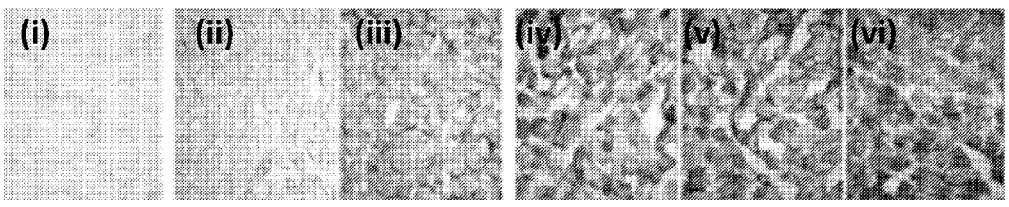

FIG. 11A

GFP

(57) Abstract: The invention provides intrathecal compositions comprising AAV particles, and their use for treating monogenic muscle disorders such as dystrophinopathies, including Duchenne muscular dystrophy.

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ADENO-ASSOCIATED VIRUS PARTICLES AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Application Serial No. 63/229,936, filed on August 5, 2021, and U.S. Provisional Application No. 63/239,881, filed on September 1, 2021, the content of each of which is incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

[0002] The invention generally relates to adeno-associated virus (AAV) particles for delivering a micro-dystrophin transgene, methods of producing the AAV particles, cells producing the AAV particles, and methods of using the AAV particles for the delivery of a micro-dystrophin transgene to skeletal and/or cardiac muscle for the treatment of dystrophinopathies, for example, Duchenne muscular dystrophy.

INCORPORATION OF THE SEQUENCE LISTING

[0003] The Sequence Listing associated with this application is provided in XML format in lieu of a paper copy and is hereby incorporated by reference into the specification. The name of the XML file containing the Sequence Listing is INMD_166_02WO_SeqList_ST26.xml. The XML file is approximately 40,532 bytes, was created on August 3, 2022, and is being submitted electronically via USPTO Patent Center.

BACKGROUND OF THE INVENTION

[0004] Duchenne muscular dystrophy (DMD) is inherited in an X-linked recessive pattern and is caused by a genetic mutation that prevents the body from producing dystrophin, a protein that muscles require to work properly. DMD is characterized in part by progressive muscle degeneration. As the disease progresses, initially affecting muscles in the thigh, pelvis, and arms, DMD eventually affects all voluntary muscles and involves the heart and breathing muscles in later stages. In Europe and North America, the prevalence of DMD is approximately 1 in 3,600 male births. DMD is the most common childhood onset form of muscular dystrophy and affects males almost exclusively. There is no known cure for DMD and current standards of treatment are primarily aimed at management of symptoms including: steroids, immunosuppressants, anticonvulsants, braces, corrective surgery, and assisted

ventilation. Aggressive management of dilated cardiomyopathy associated with DMD includes anti-congestive medications and cardiac transplantation in severe cases.

[0005] Gene therapy is a rapidly accelerating therapeutic approach wherein nucleic acids are delivered to cells harboring mutated or non-functional genes to correct the defect in the mutated cells. In certain gene therapies, nucleic acids are packaged within adeno-associated viruses (AAV) that deliver the nucleic acids to the cells. Once inside the cell nucleus, the nucleic acid then directs appropriate protein production and the virus is safely degraded. Gene therapies for the treatment of DMD have been proposed but require delivery of high systemic viral titers leading to patient toxicity, and have high manufacturing costs due to the large quantities of virus required per patient.

[0006] Delivery of micro-dystrophin (μDys), modified but functional shortened dystrophin nucleic acid sequences, in animal models and humans has been reported to promote muscle function. μDys transgenes are designed to encode various combinations of the unique functional domains of the 427 kDa dystrophin protein. μDys sequences, generally less than 5 kilobases in length, have previously been tested using AAV to deliver μDys transgenes in the murine model of DMD using the *mdx* mouse, the most widely used animal model for DMD research. The mutation in the *mdx* mouse is a nonsense point mutation (C-to-T transition) in exon 23 that aborted full-length dystrophin expression (Sicinski et al. (1989) Science 244, pp. 1578-1580, incorporated by reference herein in its entirety). Despite the promise that delivery of μDys has shown, novel therapies are needed for the treatment of DMD. The present invention addresses this and other needs.

BRIEF SUMMARY OF THE INVENTION

[0007] The present invention relates in part to adeno-associated virus (AAV) particles comprising a capsid that packages (i.e., encapsidates) a micro-dystrophin (μDys) transgene and methods for treating various dystrophinopathies with the same, for example, by intrathecal administration. In one embodiment, the μDys transgene encodes a μDys polypeptide comprising (i) an N-terminal region (NTD) comprising an actin binding site, (ii) a domain comprising three hinge regions and four spectrin repeats, and (iii) a cysteine rich domain. The μDys transgene, in one embodiment, comprises the nucleic acid sequence set forth in SEQ ID NO:5.

[0008] In one aspect, an AAV particle is provided, comprising a capsid encapsidating a vector genome. The vector genome, in one embodiment, comprises from 5' to 3': 5' inverted

terminal repeat (ITR); a promoter; a μDys transgene, an SV40 poly (A) tail; and a 3' ITR. The μDys transgene, in one embodiment, encodes a polypeptide comprising (i) an N-terminal region (NTD) comprising an actin binding site, (ii) a central rod domain comprising from two to four hinge regions and from four to six spectrin repeats, and (iii) a cysteine rich domain. In a further embodiment, the μDys transgene encodes a μDys polypeptide comprising an NTD, hinge regions 1, 2 and 4, and spectrin repeats 1, 2, 3 and 24, and a cysteine rich domain. In even a further embodiment, the μDys transgene comprises the nucleic acid sequence set forth in SEQ ID NO:5. The AAV particle, in one embodiment, is an AAV9 particle, and is present in an effective amount in an intrathecal composition. The effective amount of the AAV particle, in one embodiment, comprises about 90% or less vector genomes than the effective vector genome amount of an intravenous (IV) composition comprising an AAV particle encapsidating a μDys transgene, e.g., the same μDys transgene that is present in the intrathecal composition.

[0009] In one embodiment, the vector genome further comprises an SV40 intron 5' (upstream) of the μDys transgene and 3' (downstream) of the promoter. In another embodiment, the vector genome further comprises an enhancer is 3'(downstream) of the 5' ITR and 5' (upstream) of the promoter.

[0010] The promoter, in one embodiment, is an MHCK7 or chicken β-actin hybrid promoter.

[0011] In a preferred embodiment, the AAV particle is an AAV9 particle, i.e., the AAV particle comprises one or more AAV9 capsid proteins. The AAV9 particle's capsid, in one embodiment, consists of AAV9 capsid proteins. In yet another embodiment, the AAV particle is an AAVrh74 particle.

[0012] In some embodiments, a recombinant AAV vector genome of the present invention comprises from 5' to 3': a 5' ITR; an SK-CRM4 enhancer; a promoter; a μDys transgene; an SV40 poly (A) tail; and a 3' ITR. In some embodiments, the SK-CRM4 enhancer has a sequence comprising or consisting of SEQ ID NO:8. In some embodiments, the μDys coding sequence encodes a μDys protein comprising an actin-binding domain and at least four spectrin repeats, e.g., from four to six spectrin repeats. In some embodiments, the μDys transgene comprises or consists of the nucleic acid sequence of SEQ ID NO:5.

[0013] In some embodiments, the encapsidated vector genome of the present invention comprises a 5' AAV2 ITR and a 3' AAV2 ITR. In some embodiments, the 5' AAV2 ITR has

a sequence comprising or consisting of SEQ ID NO:1. In some embodiments, the 3' AAV2 ITR has a sequence comprising or consisting of SEQ ID NO:7.

[0014] In some embodiments, encapsidated vector genome comprises an MHCK7 promoter. In some embodiments, the MHCK7 promoter has a sequence comprising or consisting of SEQ ID NO:2. In another embodiment, the promoter is a chicken β-actin hybrid promoter. In a further embodiment, the chicken β-actin hybrid promoter has the nucleic acid sequence set forth in SEQ ID NO:3.

[0015] In some embodiments, the encapsidated vector genome of the present invention comprises an SV40 intron having a sequence comprising or consisting of SEQ ID NO:4.

[0016] In some embodiments, the encapsidated vector genome comprises a SV40 poly(A) tail having a sequence comprising or consisting of SEQ ID NO:6.

[0017] In some embodiments, the capsid of the AAV particle comprises one or more AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAVrh.74, AAV8, AAV9, AAV10, AAV11, AAV12, or AAV13 capsid proteins. In a preferred embodiment, the capsid is an AAV9 capsid and the AAV9 capsid consists of AAV9 capsid proteins.

[0018] In another aspect, the present invention relates to a method of treating a dystrophinopathy in a subject in need thereof, comprising, intrathecally administering to the subject in a single dose, a composition comprising an effective amount of one of the AAV particles encapsidating a vector genome comprising a μDys transgene, as further described herein. In one embodiment, the dystrophinopathy is Duchenne muscular dystrophy (DMD), Becker muscular dystrophy, or DMD-associated dilated cardiomyopathy (DCM). In even a further embodiment, the dystrophinopathy is DMD. The effective amount of the AAV particle, in a further embodiment, comprises about 90% or less vector genomes than the effective amount of a counterpart IV composition (e.g, an intravenously administered composition) comprising an AAV particle encapsidating a vector genome comprising a μDys transgene, e.g., the same μDys transgene that is present in the intrathecally administered composition.

[0019] In one embodiment, the subject is administered the composition when in the Trendelenburg position. In a further embodiment, administration is in the absence of a non-ionic, low-osmolar contrast agent.

[0020] In one embodiment of the method of treating a dystrophinopathy described herein, the effective dose of the intrathecally administered AAV particle provides a greater therapeutic response than the identical dose of an intravenously administered AAV particle encapsidating

a vector genome comprising a μDys transgene, e.g., the same μDys transgene that is present in the intrathecally administered AAV particle. The therapeutic response, in one embodiment, is an increase from baseline on the North Star Ambulatory Assessment (NSAA).

[0021] In another embodiment of the method of treating a dystrophinopathy described herein, intrathecal administration of an effective amount of the AAV particle encapsidating a vector genome comprising a μDys transgene, results in a decreased number of side effects, or a reduced severity of one or more side effects in the subject, compared to the number of side effects, or severity of side effects experienced by a second subject, when the second subject is intravenously administered an effective amount of a counterpart AAV particle encapsidating a vector genome comprising a μDys transgene. In a further embodiment, the dystrophinopathy is DMD. In even a further embodiment, the AAV particle is an AAV9 particle.

[0022] In even another embodiment of the method of treating a dystrophinopathy, the effective amount of the intrathecally administered AAV particle provides greater μDys transgene expression in skeletal and/or cardiac muscle, compared to the amount of μDys transgene expression in liver tissue. In a further embodiment, the dystrophinopathy is DMD. In even a further embodiment, the AAV particle is an AAV9 particle. In yet even a further embodiment, the AAV particle encapsidates a μDys transgene having the nucleic acid sequence set forth in SEQ ID NO:5.

[0023] In another aspect of the present invention, a method for preferentially delivering a μDys transgene to skeletal and/or cardiac muscle of a subject is provided. The method entails intrathecally administering to a subject in a single dose, a composition comprising an effective amount of an AAV9 particle comprising an AAV9 capsid and a vector genome comprising a μDys transgene, encapsidated by the AAV9 capsid. The encapsidated genome comprises from 5' to 3': a 5' ITR; a promoter; a μDys transgene; an SV40 poly (A) tail; and a 3' ITR. Subsequent to administration, the μDys transgene is expressed at higher levels in the skeletal and/or cardiac muscle of the subject, compared to the transgene expression in liver tissue of the subject.

[0024] In some embodiments of a method described herein, expression of a μDys transgene delivered by an AAV particle described herein in a subject is significantly less in liver tissue of the subject as compared to skeletal and/or cardiac muscle of the subject. In a further embodiment, μDys transgene expression is 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%

or at least about 80% greater in the skeletal and/or cardiac muscle of the subject compared to the amount of μDys transgene expression in the liver tissue. In another embodiment, μDys transgene expression is 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% or at least about 80% less in the liver of the subject compared to the amount of μDys transgene expression in skeletal and/or cardiac muscle.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] **FIG. 1A** shows a schematic illustration of an exemplary μDys encoding gene construct (INS1201).

[0026] **FIG. 1B** shows a schematic illustration of an alternative μDys encoding gene construct (INS1212).

[0027] **FIG. 1C** shows an agarose gel electrophoresis of the INS1201 gene construct cloned into the psZ01 vector backbone (pSZ01-INS1201) restriction digested with HindIII/BsaI, and SmaI (lanes 1 and 2, respectively), and the INS1212 gene construct cloned into the psZ01 vector backbone (pSZ01-INS1212) restriction digested with HindIII/BsaI, and SmaI (lanes 3 and 4, respectively)

[0028] **FIG. 2** shows a silver-stained SDS polyacrylamide gel electrophoresis (PAGE) of 1 μl of INS1201-AAV9 preparation (lane 1), 1 μl of INS1212-AAV9 preparation (lane 2), and 0.5 μl, 1 μl, 2 μl, and 4 μl of a 1×10^{13} vg/ml AAV2 standard (lanes 3, 4, 5, and 6, respectively).

[0029] **FIG. 3A** shows gastrocnemius muscle sections obtained from *mdx* mice 21 days post intramuscular injection with 2.7×10^{11} vg of INS1201-AAV9 (iii) or INS1212-AAV9 (iv) and immunofluorescently stained for dystrophin. Gastrocnemius sections obtained from a non-injected *mdx* mouse (i) and wildtype C57/Bl mouse (ii) and immunofluorescently stained for dystrophin are shown for comparison.

[0030] **FIG. 3B** shows a gastrocnemius muscle section obtained from an *mdx* mouse 21 days post intramuscular injection with 2.7×10^{11} vector genomes (vg) of INS1212-AAV9 and stained with DAPI (i) and for dystrophin (ii). Merged image shown in (iii).

[0031] **FIG. 4A** shows gastrocnemius (i), tibialis anterior (ii), quadriceps (iii), gluteus (iv), triceps (v), diaphragm (vi), heart (vii), and liver (viii) sections obtained from an *mdx* mouse 21 days post intracerebroventricular (ICV) injection with 2.7×10^{11} vg of INS1201-AAV9 and immunofluorescently stained for dystrophin.

[0032] **FIG. 4B** shows gastrocnemius (i), tibialis anterior (ii), quadriceps (iii), gluteus (iv), triceps (v), diaphragm (vi), heart (vii), and liver (viii) sections obtained from an *mdx* mouse 21 days post intracerebroventricular (ICV) injection with 9×10^{10} vg of INS1201-AAV9 and immunofluorescently stained for dystrophin.

[0033] **FIG. 5** shows gastrocnemius (i), tibialis anterior (ii), quadriceps (iii), gluteus (iv), triceps (v), diaphragm (vi), heart (vii), and liver (viii) sections obtained from an *mdx* mouse 21 days post intracerebroventricular (ICV) injection with 9×10^{10} vg of INS1212-AAV9 and immunofluorescently stained for dystrophin.

[0034] **FIG. 6A** shows gastrocnemius muscle sections obtained from *mdx* mice 80 days post intracerebroventricular (ICV) injection with 9×10^{10} vg (ii) or 2.7×10^{11} vg (iii) of INS1201-AAV9 and stained with hematoxylin and eosin (H&E). Gastrocnemius sections obtained from a wildtype C57/Bl mouse (i) and non-injected *mdx* mouse (iv) and H&E stained are shown for comparison.

[0035] **FIG. 6B** shows gastrocnemius muscle sections obtained from *mdx* mice 80 days post intracerebroventricular (ICV) injection with 9×10^{10} vg (ii) or 2.7×10^{11} vg (iii) of INS1201-AAV9 and stained for dystrophin. Gastrocnemius sections obtained from a wildtype C57/Bl mouse (i) and non-injected *mdx* mouse (iv) and stained for dystrophin are shown for comparison.

[0036] **FIG. 7A** shows a gastrocnemius muscle section obtained from an *mdx* mouse 80 days post intracerebroventricular (ICV) injection with 9×10^{10} vg (ii) of INS1212-AAV9 and stained with hematoxylin and eosin (H&E). Gastrocnemius sections obtained from a wildtype C57/Bl mouse (i) and non-injected *mdx* mouse (iii) and H&E stained are shown for comparison.

[0037] **FIG. 7B** shows a gastrocnemius muscle section obtained from an *mdx* mouse 80 days post intracerebroventricular (ICV) injection with 9×10^{10} vg (ii) of INS1212-AAV9 and stained for dystrophin. Gastrocnemius sections obtained from a wildtype C57/Bl mouse (i) and non-injected *mdx* mouse (iii) and stained for dystrophin are shown for comparison.

[0038] **FIG. 8A** shows a bar graph of mean fiber diameter (μm) in gastrocnemius muscle cells in an *mdx* mouse 80 days post intracerebroventricular (ICV) injection with 9×10^{10} vg or 2.7×10^{11} vg of INS1201-AAV9. Mean fiber diameters (μm) in gastrocnemius muscle cells in wildtype C57/Bl and non-injected *mdx* mice are shown for comparison.

[0039] **FIG. 8B** shows a line graph of relative frequencies (%) of cell diameters (μm) in gastrocnemius muscle cells in an *mdx* mouse 80 days post intracerebroventricular (ICV)

injection with 9×10^{10} vg or 2.7×10^{11} vg of INS1201-AAV9. Relative frequencies of cell diameters in gastrocnemius muscle cells in wildtype C57/Bl and non-injected *mdx* mice are shown for comparison.

[0040] **FIG. 8C** shows a bar graph of mean fiber diameter (μm) in triceps muscle cells in an *mdx* mouse 80 days post intracerebroventricular (ICV) injection with 9×10^{10} vg or 2.7×10^{11} vg of INS1201-AAV9. Mean fiber diameters (μm) in triceps muscle cells in wildtype C57/Bl and non-injected *mdx* mice are shown for comparison.

[0041] **FIG. 8D** shows a line graph of relative frequencies (%) of cell diameters (μm) in triceps muscle cells in an *mdx* mouse 80 days post intracerebroventricular (ICV) injection with 9×10^{10} vg or 2.7×10^{11} vg of INS1201-AAV9. Relative frequencies of cell diameters in triceps muscle cells in wildtype C57/Bl and non-injected *mdx* mice are shown for comparison.

[0042] **FIG. 8E** shows a bar graph of mean fiber diameter (μm) in tibialis anterior muscle cells in an *mdx* mouse 80 days post intracerebroventricular (ICV) injection with 9×10^{10} vg or 2.7×10^{11} vg of INS1201-AAV9. Mean fiber diameters (μm) in tibialis anterior muscle cells in wildtype C57/Bl and non-injected *mdx* mice are shown for comparison.

[0043] **FIG. 8F** shows a line graph of relative frequencies (%) of cell diameters (μm) in tibialis anterior muscle cells in an *mdx* mouse 80 days post intracerebroventricular (ICV) injection with 9×10^{10} vg or 2.7×10^{11} vg of INS1201-AAV9. Relative frequencies of cell diameters in tibialis anterior muscle cells in wildtype C57/Bl and non-injected *mdx* mice are shown for comparison.

[0044] **FIG. 8G** shows a bar graph of mean fiber diameter (μm) in diaphragm muscle cells in an *mdx* mouse 80 days post intracerebroventricular (ICV) injection with 9×10^{10} vg or 2.7×10^{11} vg of INS1201-AAV9. Mean fiber diameters (μm) in diaphragm muscle cells in wildtype C57/Bl and non-injected *mdx* mice are shown for comparison.

[0045] **FIG. 8H** shows a line graph of relative frequencies (%) of cell diameters (in μm) in diaphragm muscle cells in an *mdx* mouse 80 days post intracerebroventricular (ICV) injection with 9×10^{10} vg or 2.7×10^{11} vg of INS1201-AAV9. Relative frequencies of cell diameters in diaphragm muscle cells in wildtype C57/Bl and non-injected *mdx* mice are shown for comparison.

[0046] **FIG. 9A** shows a bar graph of mean fiber diameter (μm) in gastrocnemius muscle cells in an *mdx* mouse 80 days post intracerebroventricular (ICV) injection with 9×10^{10} vg of

INS1212-AAV9. Mean fiber diameters (μm) in diaphragm muscle cells in wildtype C57/Bl and non-injected *mdx* mice are shown for comparison.

[0047] **FIG. 9B** shows a line graph of relative frequencies (%) of cell diameters (μm) in gastrocnemius muscle cells in an *mdx* mouse 80 days post intracerebroventricular (ICV) injection with 9×10^{10} vg of INS1212-AAV9. Relative frequencies of cell diameters in diaphragm muscle cells in wildtype C57/Bl and non-injected *mdx* mice are shown for comparison.

[0048] **FIG. 10A** is a line graph of percent contractile force in EDL muscle resulting from eccentric contractions (EC) in a wildtype C57/Bl mouse, an *mdx* mouse receiving intracerebroventricular (ICV) injection at postnatal day 1 (p1) with vehicle, an *mdx* mouse receiving ICV injection at postnatal day 1 p1 of 2.7×10^{11} vg of INS1201-AAV9, and an *mdx* mouse receiving ICV injection at p1 of 9×10^{10} vg of INS1201-AAV9.

[0049] **FIG 10B** is a bar graph of percent post-eccentric contraction (EC) stress in EDL muscle relative to pre-EC stress in (i) a wildtype C57/Bl mouse, and *mdx* mice receiving intracerebroventricular (ICV) injection at postnatal day 1 (p1) of (ii) 9×10^{9} vg of INS1201-AAV9; (iii) 9×10^{10} vg of INS1201-AAV9; (iv) 2.7×10^{11} vg of INS1201-AAV9 or (v) vehicle control.

[0050] **FIG. 10C** is a graph showing the percent of contractile force in EDL muscle (% eccentric contraction 1 (EC1)) as a function of the eccentric contraction (EC) number in (i) a wildtype C57/Bl mouse, and *mdx* mice receiving intracerebroventricular (ICV) injection at postnatal day 28 (p28) of either (ii) 9×10^{10} vg of INS1201-AAV9; (iii) 2.7×10^{11} vg of INS1201-AAV9; (iv) 5.4×10^{11} vg of INS1201-AAV9; (v) 1.2×10^{12} vg of INS1201-AAV9; or (vi) vehicle control.

[0051] **FIG. 10D** is a bar graph showing the percent of force in EDL muscle ([post EC5/post EC1) in (i) a wildtype C57/Bl mouse, and *mdx* mice receiving intracerebroventricular (ICV) injection at postnatal day 28 (p28) of either (ii) 9×10^{10} vg of INS1201-AAV9; (iii) 2.7×10^{11} vg of INS1201-AAV9; (iv) 5.4×10^{11} vg of INS1201-AAV9; (v) 1.2×10^{12} vg of INS1201-AAV9; or (vi) vehicle control.

[0052] **FIG. 10E** is a graph of maximum tension (kPa) in EDL muscle resulting from eccentric contractions (EC), in (i) a wildtype C57/Bl mouse, and *mdx* mice receiving intracerebroventricular (ICV) injection at postnatal day 28 (p28) of either (ii) 9×10^{9} vg of

INS1201-AAV9; (iii) 9×10^{10} vg of INS1201-AAV9; (iv) 2.7×10^{11} vg of INS1201-AAV9; (v) 5.4×10^{11} vg of INS1201-AAV9; (vi) 1.2×10^{12} vg of INS1201-AAV9; or (vii) vehicle control.

[0053] **FIG. 10F** is a graph of peak stress (kPa) resulting from eccentric contractions (EC) at various frequencies (Hz), in (i) a wildtype C57/Bl mouse, and *mdx* mice receiving intracerebroventricular (ICV) injection at postnatal day 28 (p28) of either (ii) 9×10^{9} vg of INS1201-AAV9; (iii) 9×10^{10} vg of INS1201-AAV9; (iv) 2.7×10^{11} vg of INS1201-AAV9; (v) 5.4×10^{11} vg of INS1201-AAV9; (vi) 1.2×10^{12} vg of INS1201-AAV9; or (vii) vehicle control.

[0054] **FIG. 11A** shows gastrocnemius muscle sections obtained from non-injected cynomolgus macaques (i), cynomolgus macaques 21 days post intravenous (IV) injections with 5×10^{13} vg (ii) or 1×10^{14} vg (iii) of AAV9 CBA-GFP; or cynomolgus macaques 21 days post intrathecal (IT) injections with 2.5×10^{13} vg (iv), 5×10^{13} vg (v), or 1×10^{14} vg (vi) of AAV9 CBA-GFP and immunostained with NovaRed™ for detection of GFP expression.

[0055] **FIG. 11B** shows quadriceps muscle sections obtained from non-injected cynomolgus macaques (i), cynomolgus macaques 21 days post intravenous (IV) injections with 5×10^{13} vg (ii) or 1×10^{14} vg (iii) of AAV9 CBA-GFP; or cynomolgus macaques 21 days post intrathecal (IT) injections with 2.5×10^{13} vg (iv), 5×10^{13} vg (v), or 1×10^{14} vg (vi) of AAV9 CBA-GFP and immunostained with NovaRed™ for detection of GFP expression.

[0056] **FIG. 11C** shows deltoid muscle sections obtained from non-injected cynomolgus macaques (i); cynomolgus macaques 21 days post intravenous (IV) injections with 5×10^{13} vg (ii) or 1×10^{14} vg (iii) of AAV9 CBA-GFP; or cynomolgus macaques 21 days post intrathecal (IT) injections with 2.5×10^{13} vg (iv), 5×10^{13} vg (v), or 1×10^{14} vg (vi) of AAV9 CBA-GFP and immunostained with NovaRed™ for detection of GFP expression.

[0057] **FIG. 11D** shows triceps muscle sections obtained from non-injected cynomolgus macaques (i); cynomolgus macaques 21 days post intravenous (IV) injections with 5×10^{13} vg (ii) or 1×10^{14} vg (iii) of AAV9 CBA-GFP; or cynomolgus macaques 21 days post intrathecal (IT) injections with 2.5×10^{13} vg (iv), 5×10^{13} vg (v), or 1×10^{14} vg (vi) of AAV9 CBA-GFP and immunostained with NovaRed™ for detection of GFP expression.

[0058] **FIG. 11E** shows biceps muscle sections obtained from non-injected cynomolgus macaques (i); cynomolgus macaques 21 days post intravenous (IV) injections with 5×10^{13} vg (ii) or 1×10^{14} vg (iii) of AAV9 CBA-GFP; or cynomolgus macaques 21 days post intrathecal (IT) injections with 2.5×10^{13} vg (iv), 5×10^{13} vg (v), or 1×10^{14} vg (vi) of AAV9 CBA-GFP and immunostained with NovaRed™ for detection of GFP expression.

[0059] **FIG. 11F** shows tibialis anterior muscle sections obtained from cynomolgus macaques 21 days post intravenous (IV) injections with 5×10^{13} vg (i) or 1×10^{14} vg (ii) of AAV9 CBA-GFP; or cynomolgus macaques 21 days post intrathecal (IT) injections with 2.5×10^{13} vg (iii), 5×10^{13} vg (iv), or 1×10^{14} vg (v) of AAV9 CBA-GFP and immunostained with NovaRed™ for detection of GFP expression.

[0060] **FIG. 11G** shows diaphragm muscle sections obtained from cynomolgus macaques 21 days post intravenous (IV) injections with 5×10^{13} vg (i) or 1×10^{14} vg (ii) of AAV9 CBA-GFP; or cynomolgus macaques 21 days post intrathecal (IT) injections with 2.5×10^{13} vg (iii), 5×10^{13} vg (iv), or 1×10^{14} vg (v) of AAV9 CBA-GFP and immunostained with NovaRed™ for detection of GFP expression.

[0061] **FIG. 11H** shows heart muscle sections obtained from cynomolgus macaques 21 days post intravenous (IV) injections with 5×10^{13} vg (i) or 1×10^{14} vg (ii) of AAV9 CBA-GFP; or cynomolgus macaques 21 days post intrathecal (IT) injections with 2.5×10^{13} vg (iii), 5×10^{13} vg (iv), or 1×10^{14} vg (v) of AAV9 CBA-GFP and immunostained with NovaRed™ for detection of GFP expression.

[0062] **FIG. 11I** shows liver sections obtained from cynomolgus macaques 21 days post intravenous (IV) injections with 5×10^{13} vg (i) or 1×10^{14} vg (ii) of AAV9 CBA-GFP; or cynomolgus macaques 21 days post intrathecal (IT) injections with 2.5×10^{13} vg (iii), 5×10^{13} vg (iv), or 1×10^{14} vg (v) of AAV9 CBA-GFP and immunostained with NovaRed™ for detection of GFP expression.

[0063] **FIG. 12A** shows a Ponceau stain (top panel) or Western Blot (bottom panel) of biceps (1), triceps (2), deltoid (3), quadriceps (4), gastrocnemius (5), tibialis anterior (6), diaphragm (7), and heart (8) muscle protein samples obtained from cynomolgus macaques 21 days post intrathecal (IT) injections with 2.5×10^{13} vg of AAV9 CBA-GFP and probed with anti-GFP antibody.

[0064] **FIG. 12B** shows a Ponceau stain (top panel) or Western Blot (bottom panel) of biceps (1), triceps (2), deltoid (3), quadriceps (4), gastrocnemius (5), tibialis anterior (6), diaphragm (7), and heart (8) muscle protein samples obtained from cynomolgus macaques 21 days post intrathecal (IT) injections with 5×10^{13} vg of AAV9 CBA-GFP and probed with anti-GFP antibody.

[0065] **FIG. 12C** shows a Ponceau stain (top panel) or Western Blot (bottom panel) of biceps (1), triceps (2), deltoid (3), quadriceps (4), gastrocnemius (5), tibialis anterior (6),

diaphragm (7), and heart (8) muscle protein samples obtained from cynomolgus macaques 21 days post intrathecal (IT) injections with 1×10^{14} vg of AAV9 CBA-GFP and probed with anti-GFP antibody.

[0066] **FIG. 12D** shows a Ponceau stain (top panel) or Western Blot of biceps (1), and triceps (2) muscle protein samples obtained from non-injected cynomolgus macaques and probed with anti-GFP antibody.

[0067] **FIG. 13** shows an agarose gel electrophoresis of biceps (1), triceps (2), deltoid (3), tibialis anterior (4), gastrocnemius (5), quadriceps vastus lateralis (6), diaphragm (7), and heart (8) muscle and liver (9) tissue samples obtained from cynomolgus macaques 21 days post intrathecal (IT) injections with 2.5×10^{13} vg of AAV9 CBA-GFP and subjected to RT-PCR in the presence (+) or absence (-) of reverse transcriptase. Biceps (10), triceps (11), deltoid (12), and quadriceps (13) muscle tissue samples obtained from non-injected cynomolgous macaques and subjected to RT-PCR in the presence (+) or absence (-) of reverse transcriptase are shown for comparison.

[0068] **FIG. 14** shows various μDys protein domains encoded by μDys transgenes provided herein.

[0069] **FIG. 15** is a graph showing the number of INS1201 DNA copies per diploid genome in *mdx* mice receiving intracerebroventricular (ICV) injection at postnatal day 28 (p28) of either (ii) 9×10^{9} vg of INS1201-AAV9; (iii) 9×10^{10} vg of INS1201-AAV9; (iv) 2.7×10^{11} vg of INS1201-AAV9; (v) 5.4×10^{11} vg of INS1201-AAV9; (vi) 1.2×10^{12} vg of INS1201-AAV9; or (vii) vehicle control.

[0070] **FIG. 16** is a graph showing the number of INS1201 RNA transcript copies normalized to the number of copies of RPP30 in *mdx* mice receiving intracerebroventricular (ICV) injection at postnatal day 28 (p28) of either (ii) 9×10^{9} vg of INS1201-AAV9; (iii) 9×10^{10} vg of INS1201-AAV9; (iv) 2.7×10^{11} vg of INS1201-AAV9; (v) 5.4×10^{11} vg of INS1201-AAV9; (vi) 1.2×10^{12} vg of INS1201-AAV9; or (vii) vehicle control.

[0071] **FIG. 17** is a graph of mean fiber diameter (μm) in EDL muscle cells at postnatal day 120 (p120) in (i) wildtype C57/Bl mice, and *mdx* mice receiving intracerebroventricular (ICV) injection at postnatal day 28 (p28) of either (ii) 9×10^{9} vg of INS1201-AAV9; (iii) 9×10^{10} vg of INS1201-AAV9; (iv) 2.7×10^{11} vg of INS1201-AAV9; (v) 5.4×10^{11} vg of INS1201-AAV9; (vi) 1.2×10^{12} vg of INS1201-AAV9; or (vii) vehicle control.

[0072] **FIG. 18** is a graph of mean fiber diameter (μm) in tibialis anterior muscle cells at postnatal day 120 (p120) in (i) wildtype C57/Bl mice, and *mdx* mice receiving intracerebroventricular (ICV) injection at postnatal day 28 (p28) of either (ii) 9×10^{9} vg of INS1201-AAV9; (iii) 9×10^{10} vg of INS1201-AAV9; (iv) 2.7×10^{11} vg of INS1201-AAV9; (v) 5.4×10^{11} vg of INS1201-AAV9; (vi) 1.2×10^{12} vg of INS1201-AAV9; or (vii) vehicle control.

[0073] **FIG. 19** shows diaphragm muscle sections stained with picrosirius red, post intracerebroventricular (ICV) injection with various doses of INS1201-AAV9. Diaphragm sections obtained from a wildtype C57/Bl mouse and *mdx* mouse administered vehicle are shown for comparison in the top section.

[0074] **FIG. 20** is a graph showing the percent collagen in diaphragm muscle at postnatal day 120 (p120), in (i) wildtype C57/Bl mice, and *mdx* mice receiving intracerebroventricular (ICV) injection at postnatal day 28 (p28) of either (ii) vehicle control; (iii) 9×10^{9} vg of INS1201-AAV9; (iv) 9×10^{10} vg of INS1201-AAV9; (v) 2.7×10^{11} vg of INS1201-AAV9; (v) 5.4×10^{11} vg of INS1201-AAV9 or (vi) 1.2×10^{12} vg of INS1201-AAV9.

[0075] **FIG. 21**, top, shows an extensor digitorum longus (EDL) muscle section obtained from an *mdx* mouse at postnatal day 120 (p120) subsequent to intracerebroventricular (ICV) injection at p28 with 5.4×10^{11} vg of INS1201-AAV9 and stained with hematoxylin and eosin (H&E) (far left); laminin/dapi (second from left); dystrophin (second from right); and a merged image (far right). **FIG. 21**, bottom, shows a EDL muscle section obtained from an *mdx* mouse at postnatal day 120 (p120) subsequent to intracerebroventricular (ICV) injection at p28 with 5vehicle control, and stained with hematoxylin and eosin (H&E) (far left); laminin/dapi (second from left); dystrophin (second from right); and a merged image (far right).

DETAILED DESCRIPTION OF THE INVENTION

[0076] The present invention relates in part to adeno-associated viral (AAV) particles and methods for preferentially delivering the same to cardiac and/or skeletal muscle to a subject in need of treatment of a monogenic muscle disease, for example, a dystrophinopathy such as Duchenne muscular dystrophy (DMD), Becker muscular dystrophy, or DMD-associated dilated cardiomyopathy (DCM). Without wishing to be bound by theory, the particles and methods for delivering the same to subjects in need thereof, for example to treat a monogenic muscle disease such as a dystrophinopathy, provide a superior benefit to known AAV particles and treatment methods at least because the present invention: (i) allows for significantly lower dosages than IV delivery, in order to achieve substantially the same or a better therapeutic

benefit, thereby reducing viral load and toxicity and other side effects; and/or (ii) allows for preferential transgene targeting and expression in cardiac and/or skeletal muscle tissue compared to liver tissue, thereby targeting the transgene to cells of interest to provide a greater therapeutic benefit compared to AAV vectors delivered intravenously; (iii) can benefit greater patient populations compared to IV formulations, because of the lower dosages needed, corresponding to a decreased manufacturing burden.

[0077] Aspects of the invention relate to AAV particles, methods for producing the same, and methods for delivering the AAV particles to a subject in need of treatment. The AAV particle for example, an AAV9 particle, comprises a capsid comprising one or more AAV9 capsid proteins and a vector genome encapsidated by the AAV9 capsid. The vector genome comprises a transgene and regulatory elements that promote gene expression of the transgene when delivered into muscle cells, for example skeletal and/or cardiac muscle cells. In one embodiment, the transgene is a micro-dystrophin (μDys) transgene.

[0078] Methods described herein comprise intrathecally administering to a subject in need of treatment of a dystrophinopathy, in a single dose, a composition comprising an effective amount of an AAV particle as described herein. The dystrophinopathy in one embodiment, is DMD, Becker muscular dystrophy, or DCM. In a preferred embodiment, the dystrophinopathy is DMD. In embodiments described herein, intrathecal delivery of AAV particles of the present invention to muscle cells can result in robust expression of μDys and can significantly improve muscle health and function. The present invention also provides methods and cells for producing the AAV particles described herein. In one embodiment of the methods described herein, subsequent to intrathecal administration of the AAV particle, the transgene is expressed at higher levels in the skeletal and/or cardiac muscle of the subject, compared to the transgene expression in liver tissue of the subject.

[0079] To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

[0080] The terms “a” and “an” as used herein mean “one or more” and include the plural unless the context is inappropriate.

[0081] The term “nucleic acid,” “nucleotide,” or “oligonucleotide” refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties

as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka *et al.*, J. Biol. Chem. 260:2605-2608 (1985); and Rossolini *et al.*, Mol. Cell. Probes 8:91-98 (1994)).

[0082] The term "gene" can refer to the segment of DNA involved in producing or encoding a polypeptide chain. It may include regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons). Alternatively, the term "gene" can refer to the segment of DNA involved in producing or encoding a non-translated RNA, such as an rRNA, tRNA, guide RNA (gRNA), short-interfering RNA (siRNA), or micro RNA (miRNA).

[0083] As used herein, the term "transgene" refers to an exogenous gene present in a vector genome, artificially introduced into the genome of a cell, or an endogenous gene artificially introduced into a non-natural locus in the genome of a cell. A transgene can refer to a segment of DNA involved in producing or encoding a polypeptide chain. Transgenes may include regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons). The transgene according to embodiments described herein, is μDys. The μDys transgene, in one embodiment, encodes a μDys polypeptide comprising an N-terminal region, from about two to three hinge regions, from about four to six spectrin repeats, and a cysteine rich domain.

[0084] "Vector genome" as used herein, is a nucleic acid genome comprising one or more heterologous nucleic acid sequences. The one or more heterologous nucleic acid sequences comprises a transgene. In some embodiments of the invention, the vector genome comprises at least one ITR sequence (e.g., AAV ITR sequence), optionally two ITRs (e.g., two AAV ITRs), which typically will be at the 5' and 3' ends of the vector genome and flank the one or more heterologous nucleic acids. The ITRs can be the same or different from each other.

[0085] As used herein, the term "endogenous" with reference to a nucleic acid, for example, a gene, or a protein in a cell, is a nucleic acid or protein that occurs in that particular

cell as it is found in nature, for example, at its natural genomic location or locus. Moreover, a cell "endogenously expressing" a nucleic acid or protein expresses that nucleic acid or protein as it is found in nature.

[0086] A "promoter" is defined as one or more nucleic acid control sequence(s) that direct transcription of a nucleic acid, e.g., a transgene, and can be present within a vector genome. As used herein, a promoter includes nucleic acid sequences near the start site of transcription. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription.

[0087] A "regulatory element" as used herein refers to a nucleic acid sequence capable of regulating transcription of a gene (e.g., a transgene), and/or regulate the stability or translation of a transcribed mRNA product, and can be present within a vector genome. In some embodiments, regulatory elements can regulate tissue-specific transcription of a gene. Regulatory elements can comprise at least one transcription factor binding site, for example, a transcription factor binding site for a muscle-specific transcription factor. Regulatory elements as used herein increase or enhance promoter-driven gene expression when compared to the transcription of the gene from the promoter alone in the absence of the regulatory element. Regulatory elements as used herein may occur at any distance (i.e., proximal or distal) to the transgene they regulate. Regulatory elements as used herein may comprise part of a larger sequence involved in transcriptional control, e.g., part of a promoter sequence. However, regulatory elements alone are typically not sufficient to initiate transcription on its own and require the presence of a promoter.

[0088] A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation.

[0089] "Polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. As used herein, the terms encompass amino acid chains of any length, including full-length proteins, and functional fragments thereof, wherein the amino acid residues are linked by covalent peptide bonds.

[0090] As used herein, the term "complementary" or "complementarity" refers to specific base pairing between nucleotides or nucleic acids. Complementary nucleotides are, generally, A and T (or A and U), and G and C. The guide RNAs (gRNAs) described herein can comprise

sequences, for example, DNA targeting sequences that are perfectly complementary or substantially complementary (e.g., having a small fraction of mismatched bases) to a genomic sequence.

[0091] As used herein, the terms "introducing" or "delivering" in the context of nucleic acids, for example, AAV vectors, refers to the translocation of the nucleic acid from outside a cell to inside the cell, for example, a muscle cell. In some cases, introducing refers to translocation of the nucleic acid from outside the cell to inside the nucleus of the cell. Various methods of such translocation are contemplated, including but not limited to, electroporation, contact with nanowires or nanotubes, receptor mediated internalization, translocation via cell penetrating peptides, liposome-mediated translocation, and the like.

[0092] As used herein, the terms "packaged" or "encapsidated" refers to the inclusion of a vector genome in a capsid comprising viral capsid proteins to form an AAV particle.

[0093] The term "substantial identity" or "substantially identical," as used in the context of polynucleotide or polypeptide sequences, refers to a sequence that has at least 60% sequence identity to a reference sequence. Alternatively, percent identity can be any integer from 60% to 100%. Exemplary embodiments include at least: 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, as compared to a reference sequence using the programs described herein; preferably BLAST using standard parameters, as described below. One of skill will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

[0094] For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0095] Algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-410 and Altschul *et al.* (1977) *Nucleic Acids Res.* 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the

National Center for Biotechnology Information (NCBI) web site. The algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al, supra*). These initial neighborhood word hits acts as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word size (W) of 28, an expectation (E) of 10, M=1, N=-2, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word size (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)).

[0096] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.01, more preferably less than about 10^{-5}, and most preferably less than about 10^{-20}.

[0097] As used herein, the terms "subject" and "patient" refer to an organism to be treated by the methods and compositions described herein. Such organisms include, but are not limited to, mammals such as humans, simians, murines, equines, bovines, porcines, canines, felines, and the like. In some embodiments, the subject or patient is human. The subject in the methods of treatment provided herein, in one embodiment, is a male subject.

[0098] In embodiments where the subject is a male human, the male human subject is from about 4 years old to about 7 years old, a newborn, about 1 year to about 7 years old, about 2 years to about 7 years old, about 2 years to about 6 years old, about 2 years to about 5 years old, about 2 years to about 4 years old, about 3 years to about 7 years old, about 3 years to about 6 years old, about 1 month to about 6 years old, about 1 month to about 5 years old, about 1 month to about 4 years old, about 1 month to about 3 years old, about 1 month to about 2 years old, or about 1 month to about 12 months old.

[0099] In one embodiment, the subject is a male human patient from about 4 years old to about 7 years old. In one embodiment, the subject is a male human patient from about 3 years old to about 7 years old. In one embodiment, the subject is a male human patient from about 2 years old to about 7 years old.

[0100] As used herein, the term "efficient delivery" or "efficiently delivering" refers to administration of an AAV particle comprising an AAV capsid encapsidating a vector genome encoding a transgene that results in expression of the transgene in a desired cell or tissue.

[0101] As used herein, the term "effective amount" or "effective dose", refers to the amount of a substance (e.g., an AAV particle of the present invention) sufficient to effect beneficial or desired results (e.g., expression of a protein, or a desired prophylactic or therapeutic effect). An effective amount can be administered in one or more administration(s), application(s) or dosage(s) and is not intended to be limited to a particular formulation or administration route. Where a dose is provided in "vector genomes", an "effective dose" may be referred to herein as an "effective vector genome dose".

[0102] As used herein, the term "treating" includes any effect, *e.g.*, lessening, reducing, modulating, ameliorating or eliminating, that results in the improvement of the condition, disease, disorder, and the like, or ameliorating a symptom thereof.

[0103] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

Adeno-Associated Virus (AAV) Particles

[0104] As described herein, one aspect of the present invention relates to an AAV particle comprising one or more AAV capsid proteins and a vector genome encapsidated by the one or more capsid proteins; and intrathecal compositions comprising the same. The genome comprises from 5' to 3', a 5' inverted terminal repeat (ITR), a promoter, a transgene, an SV40 poly (A) tail; and a 3' ITR. When intrathecally administered to subjects in need of treatment, in one embodiment, the transgene is expressed at higher levels in the skeletal and/or cardiac muscle of the subject, compared to the transgene expression in liver tissue of the subject. In another embodiment, the ratio of [(skeletal and/or cardiac muscle transgene expression)] / (liver transgene expression)] provided by an AAV particle described herein, is greater than the same ratio when the identical dose of the same AAV particle is administered intravenously. In one preferred embodiment, the AAV particle is an AAV9 particle comprising one or more AAV9 capsid proteins.

[0105] As used herein, an "adeno-associated virus (AAV) particle", refers to an AAV virion comprising an AAV capsid and a vector genome encapsidated by the AAV capsid. The vector genome typically comprises a promoter and one or more transgenes, that are flanked by AAV ITR sequences. The AAV capsid comprises one or more AAV capsid proteins. The AAV capsid proteins can be from the same or different AAV serotypes and can be wild-type or engineered. Vector genomes described herein can be replicated, and packaged into viral vectors (particles) when introduced into a host cell also comprising one or more plasmids encoding *rep* and *cap* gene products. In one embodiment, a helper plasmid is also transfected into the host cell to aid in vector production by the host cell. In one embodiment, the AAV vector used herein is an AAV9 vector, for example, as described in U.S. Patent No. 7,906,111, the disclosure of which is incorporated by reference herein in its entirety for all purposes. In one embodiment, the AAV capsid is an AAV9 capsid.

[0106] The terms "empty capsid," "empty vial particle," and "empty AAV" refer to an AAV capsid shell lacking a vector genome packaged within.

[0107] Encapsidated vector genomes described herein can include one or more regulatory elements, for example one or more regulatory elements upstream of the transgene. The encapsidated genome, in one embodiment, comprises from 5' to 3', a 5' ITR, a promoter, a transgene, an SV40 poly (A) tail; and a 3' ITR. In another embodiment, the encapsidated genome comprises from 5' to 3', a 5' ITR, an enhancer, a promoter, a transgene, an SV40 poly

(A) tail; and a 3' ITR. The encapsidated genome, in even another embodiment, comprises from 5' to 3', a 5' ITR, a promoter, a SV40 intron, a transgene, an SV40 poly (A) tail; and a 3' ITR. In yet even another embodiment, the encapsidated genome comprises from 5' to 3', a 5' ITR, an enhancer, a promoter, an SV40 intron, a transgene, an SV40 poly (A) tail; and a 3' ITR.

Inverted Terminal Repeats

[0108] Inverted terminal repeats (ITR) are palindromic 145 nucleotide sequences that flank a transgene. The 5' and 3' ITRs of an AAV vector genome are necessary for both the integration of the transgene into the host cell genome (*e.g.*, chromosome 19 in humans) and for encapsidation of the transgene into the AAV particle.

[0109] In some embodiments, AAV vector genomes of the present invention comprise ITR sequences from any one AAV serotype, for example, AAVrh.74, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV8, AAV9, AAV10, AAV11, AAV12, or AAV13. In some embodiments, the AAV vector genomes disclosed herein comprise a 5' AAV2 ITR and a 3' AAV2 ITR sequence.

[0110] In some embodiments, AAV vector genomes described herein comprise a 5' AAV2 ITR having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:1 (*see* Table 1). In some embodiments, the 5' AAV2 ITR comprises SEQ ID NO:1. In some embodiments, the 5' AAV2 ITR consists of SEQ ID NO:1.

[0111] In some embodiments, AAV vector genomes described herein comprise a 3' AAV2 ITR having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:7 (*see* Table 1). In some embodiments, the 3' AAV2 ITR comprises SEQ ID NO:7. In some embodiments, the 3' AAV2 ITR consists of SEQ ID NO:7.

Promoters

[0112] Promoters drive the expression of the transgene and are typically located upstream (or 5') of the transgene whose expression they regulate.

[0113] In some embodiments, AAV vector genomes of the present invention comprise a mammalian promoter, for example, human, non-human primate (*e.g.*, cynomolgous macaque), mouse, horse, cow, pig, cat, and dog promoters. In some embodiments, recombinant AAV vector genomes disclosed herein comprise strong, constitutively active promoters to drive high-

level expression of the transgene. For example, in some embodiments, the promoter is a cytomegalovirus (CMV) promoter/enhancer, an elongation factor 1α (EF1α) promoter, a simian virus 40 (SV40) promoter, a chicken β-actin hybrid promoter, or a CAG promoter.

[0114] The promoter, in one embodiment, is an MHCK7 or chicken β-actin hybrid promoter.

[0115] In certain embodiments, an AAV vector genome of the present invention comprise a muscle-specific promoter that is operably linked to the transgene to drive high-level and tissue-specific expression in muscle cells. For example, muscle specific promoters of the present invention include, but are not limited to, promoters selected from: desmin (DES, also known as CSM1 or CSM2) promoter, the alpha 2 actinin (ACTN2, also known as CMD1AA) promoter, the filamin-C (FLNC, also known as actin-binding-like protein (ABLP), filamin-2 (FLN2), ABP-280, ABP280A, ABPA, ABPL, MFM5 or MPD4) promoter, the sarcoplasmic/endoplasmic reticulum calcium ATPase 1 (ATP2A1, also known as ATP2A or SERCA1) promoter, the troponin I type 1 (TNNI1, also known as SSTNI or 25TTNI) promoter, the myosin-1 (MYH1) promoter, the phosphorylatable, fast skeletal muscle myosin light chain (MYLPF) promoter, myosin 1 (MYH1, also known as MYHSA1, MYHa, MyC-2X/D or MyHC-2x) promoter, the alpha-3 chain tropomyosin (TPM3, also known as CFTD, NEM1, OK/Scl.5, TM-5, TM3, TM30, TM30nm, TM5, TPMsk3, TRK, h TM5 or hscp30) promoter, the ankyrin repeat domain-containing protein 2 (ANKRD2, also known as ARPP) promoter, the myosin heavy-chain (MHC) promoter, the myosin light-chain (MLC) promoter, the muscle creatine kinase (MCK) promoter, synthetic muscle promoters as described in Li et al. (1999. Nat Biotechnol. 17:241-245), such as the SPc5-12 promoter, the muscle creatine kinase (MCK) promoter, the dMCK promoter, the tMCK promoter consisting of respectively, a double or triple tandem of the MCK enhancer to the MCK basal promoter as described in Wang et al. (2008. Gene Ther. 15:1489-1499) and hybrid promoters such as the hybrid alpha-myosin heavy chain enhancer/MCK enhancer (MHCK7; 770 bp); the MCK-C5-12 promoter as described in Wang et al. (2008. Gene Ther. 15:1489-1499) and the cardiac and skeletal muscle-specific myosin chaperone Unc45b (195 bp) promoter as described in Rudeck S et al. (2016, Genesis. 54(8): 431-8). Non-limiting examples of heart-specific promoters include the calsequestrin 2 (also known as PDIB2, FLJ26321, FLJ93514 or CASQ2 (GeneID 845 for the human gene)) promoter, the ankyrin repeat domain 1 (also known as cardiac ankyrin repeat protein) promoter, the cytokine inducible nuclear protein promoter; the liver ankyrin repeat domain 1 (ANKRD1; GeneID 27063 for the human gene) promoter; the myosin, light chain 2, regulatory, cardiac,

slow (MYL2; GeneID 4633 for the human gene) promoter; the myosin, light chain 3, alkali; ventricular, skeletal 10 slow (MYL3; GeneID 4634 for the human gene) promoter; the bromodomain containing 7 (also known as BP75, CELTIX1, NAG4(BRD7; GeneID 29117 for the human gene)) promoter; the alpha myosin heavy chain (αMHC) promoter; the cardiac troponin C promoter and the promoter of the cardiac sodium-calcium exchanger (NCX1), which confers cardiac specificity.

[0116] In some embodiments, AAV vector genomes described herein comprise a MHCK7 promoter. For example, in some embodiments the MHCK7 promoter has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:2 (see Table 1). In some embodiments, the MHCK7 promoter comprises SEQ ID NO:2. In some embodiments, the MHCK7 promoter consists of SEQ ID NO:2.

SV40 Intron

[0117] In some embodiments, AAV vector genomes of the present invention comprise an SV40 intron. The SV40 intron is a commonly used regulatory element in gene therapy vectors and enhances translation and stability of the expressed RNA transcript.

[0118] In certain embodiments, the SV40 intron is downstream (i.e., 3') of the promoter and upstream (i.e., 5') of the transgene. In other embodiments, the SV40 intron can be downstream (i.e., 3') of the transgene.

[0119] In some embodiments, the SV40 intron has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:4 (see Table 1). In some embodiments, the SV40 intron comprises SEQ ID NO:4. In some embodiments, the SV40 intron consists of SEQ ID NO:4.

SV40-poly(A) Tail

[0120] In some embodiments, AAV vector genomes of the present invention comprise a nucleic acid sequence encoding an SV40 poly(A) tail. The SV40-poly(A) tail sequence is a commonly used nucleic acid element in gene therapy vectors that assists in RNA export from the nucleus, translation of RNA, and RNA stability.

[0121] In some embodiments, AAV vector genomes of the present invention comprise a sequence encoding an SV40 poly(A) tail having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:6 (see Table 1).

In some embodiments, the sequence encoding the SV40 poly(A) tail comprises SEQ ID NO:6. In some embodiments, the sequence encoding the SV40 poly(A) tail consists of SEQ ID NO:6.

Enhancers

[0122] In some embodiments, AAV vector genomes of the present invention comprise one or more enhancer sequence(s). An enhancer sequence, in one embodiment, can increase the level of transcription of the transgene, for example, by serving as a binding site for transcription factors and co-regulators that assist in DNA looping and recruitment of the transcriptional machinery to promoters.

[0123] In some embodiments, the enhancer is downstream (i.e., 3') of the 5' ITR and upstream (i.e., 5') of the promoter. In some embodiments, the enhancer is downstream (i.e., 3') of the promoter and upstream (i.e., 5') of the transgene. In some embodiments, the enhancer is downstream (i.e., 3') of the transgene and upstream (i.e., 5') of the 3' UTR.

[0124] In some embodiments, recombinant AAV vector genomes of the present invention comprise an enhancer that significantly promotes the transcription of a transgene in muscle cells, e.g., skeletal and/or cardiac muscle cells.

[0125] In some embodiments, recombinant AAV vectors described herein comprise a skeletal-cis-regulatory module 4 (SK-CRM4) enhancer. For example, in some embodiments the SK-CRM4 enhancer has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:8 (see Table 1). In some embodiments, the SK-CRM4 enhancer comprises SEQ ID NO:8. In some embodiments, the SK-CRM4 enhancer consists of SEQ ID NO:8.

[0126] In yet another embodiment, an AAV vector genome of the present invention comprises a cytomegalovirus (CMV) enhancer nucleic acid sequence. In a further embodiment, the CMV enhancer is upstream of a promoter sequence. For example, in one embodiment, the CMV enhancer has the nucleic acid sequence of SEQ ID NO:9. In some embodiments, the CMV enhancer has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:9 (*see* Table 1). In some embodiments, the CMV enhancer comprises SEQ ID NO:9. In some embodiments, the CMV enhancer consists of SEQ ID NO:9.

Transgene

[0127] Transgenes for use with the present invention are nucleic acid sequences encoding a polypeptide, or functional fragment thereof, to be expressed in a cell (*e.g.*, a muscle cell) into which the transgene is delivered.

[0128] In some embodiments, the transgene may be incorporated into the genome of the host cell to which it is delivered or may be expressed episomally.

[0129] In some embodiments, a transgene can encode a polypeptide, or functional fragment thereof, that is not endogenously expressed by the cell in which the transgene is delivered. In some embodiments, the transgene encodes a mutant form of a polypeptide, or functional fragment thereof, that is endogenously expressed by the cell in which the transgene is delivered. In some embodiments, the transgene encodes a protein, or functional fragment thereof, that is endogenously expressed by the cell in which the transgene is delivered but is expressed at low levels, and wherein expression of the transgene results in higher expression levels of the protein, or functional fragment thereof. In some embodiments, the cell in which the transgene is delivered harbors one or more mutation(s) that results in lowered levels of expression of an endogenous protein and/or a functionally defective protein, and wherein expression of the transgene results in restored expression of the endogenous protein and/or functional supplementation of the defective protein. In some embodiments, the transgene is silent when introduced into the cell in which the transgene is delivered and expression may be induced.

[0130] In some embodiments, the transgene may be heterologous (i.e., from a different species) or homologous (i.e., from the same species) to the promoter and/or other regulatory elements present in the recombinant AAV vectors described herein. In some embodiments, the transgene may be heterologous or homologous to the cell into which the transgene is delivered.

[0131] In some embodiments, the transgene may be a full length cDNA or genomic DNA sequence, or a fragment or mutant thereof having functional activity. In some embodiments, the transgene may be a minigene, i.e., a gene sequence lacking part, most or all of its intronic sequences or may contain all of its intron sequences. In some embodiments, the transgene may be a hybrid nucleic acid sequence comprising homologous and/or heterologous cDNA and/or genomic DNA fragments. In some embodiments, the transgene may comprise one or more nucleotide substitutions, deletions, and/or insertions as compared to a wildtype sequence.

[0132] In some embodiments, transgenes of the present invention encode a therapeutic protein. In certain embodiments, the transgene may encode a structural protein.

[0133] In some embodiments, the transgene is a micro-dystrophin (μDys) transgene that encodes a μDys polypeptide. In a further embodiment, the μDys polypeptide encoded by the transgene comprises (i) an N-terminal region (NTD) comprising an actin binding site, (ii) a central rod domain comprising from two to four hinge regions and from four to six spectrin repeats. In a further embodiment, the μDys transgene comprises the nucleic acid sequence set forth in SEQ ID NO:5. In even a further embodiment, the μDys transgene consists of the nucleic acid sequence set forth in SEQ ID NO:5. In another embodiment, the sequence encoding a μDys protein has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:5.

[0134] The μDys transgene in one embodiment, comprises the nucleic acid sequence set forth at SEQ ID NO:4 of U.S. Patent No. 10,351,611, incorporated by reference herein in its entirety for all purposes. In another embodiment, the sequence encoding a μDys protein has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:4 of U.S. Patent No. 10,351,611.

[0135] In one embodiment, the μDys transgene encodes a μDys polypeptide comprising (i) an N-terminal region (NTD) comprising an actin binding site, (ii) a domain comprising three hinge regions and four spectrin repeats, and (iii) a cysteine rich domain. The μDys transgene, in a further embodiment, encodes a μDys polypeptide comprising an N-terminal region (NTD) comprising an actin binding site, (ii) a central rod domain comprising hinge regions 1, 2 and 4, and spectrin repeats 1, 2, 3 and 24, and a (iii) cysteine rich domain.

[0136] The μDys transgene, in one embodiment, encodes dystrophin spectrin repeats 16 and 17, which have been reported as a scaffold for sarcolemmal neuronal nitric oxide synthase (nNOS) targeting. In a further embodiment, the μDys transgene encodes dystrophin spectrin repeats 1 and 24. In another embodiment, the μDys transgene encodes dystrophin spectrin repeats 1, 16 and 17, 23 and 24. In yet another embodiment, the μDys transgene encodes dystrophin spectrin repeats 1, 2, 3 and 24. In yet even another embodiment, the μDys transgene encodes dystrophin spectrin repeats 1, 2, 22, 23 and 24.

[0137] The μDys transgene, in one embodiment, encodes dystrophin hinge regions 1 and 4. In another embodiment, the μDys transgene encodes dystrophin hinge regions 1, 3 and 4.

[0138] In one embodiment, the μDys transgene encodes a μDys protein comprising one of the combinations of μDys domains set forth in **FIG. 14**.

[0139] The AAV vector genomes described herein comprise a μDys transgene encoding a μDys protein comprising (i) an NTD comprising an actin binding site, (ii) a central rod domain comprising from two to four hinge regions and from four to six spectrin repeats, and (iii) a cysteine rich domain. For example, in one embodiment, the μDys transgene encodes dystrophin spectrin repeats 16 and 17. In a further embodiment, the μDys transgene encodes dystrophin spectrin repeats 1 and 24. In another embodiment, the μDys transgene encodes dystrophin spectrin repeats 1, 16 and 17, 23 and 24. In yet another embodiment, the μDys transgene encodes dystrophin spectrin repeats 1, 2, 3 and 24. In yet even another embodiment, the μDys transgene encodes dystrophin spectrin repeats 1, 2, 22, 23 and 24.

[0140] The μDys transgene in one embodiment, encodes dystrophin hinge regions 1 and 4. In another embodiment, the μDys transgene encodes dystrophin hinge regions 1, 3 and 4.

[0141] AAV particles described herein, in one embodiment, comprise an encapsidated transgene encoding a μDys protein. For example, in some embodiments the sequence encoding a μDys protein has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:5 (see Table 1). In some embodiments, the sequence encoding a μDys protein comprises SEQ ID NO:5. In some embodiments, the sequence encoding a μDys protein consists of SEQ ID NO:5.

Table 1: Exemplary vector genome components for use with the present invention.

Regulatory Element or Transgene	**Sequence**
5' AAV2 ITR	CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGG GCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAG CGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCT [SEQ ID NO:1]
MHCK7 promoter	CCTTCAGATTAAAAATAACTGAGGTAAGGGCCTGGGTAGGGGAG GTGGTGTGAGACGCTCCTGTCTCTCCTCTATCTGCCCATCGGCCC TTTGGGGAGGAGGAATGTGCCCAAGGACTAAAAAAAGGCCATG GAGCCAGAGGGGCGAGGGCAACAGACCTTTCATGGGCAAACCTT GGGGCCCTGCTGTCTAGCATGCCCCACTACGGGTCTAGGCTGCCC ATGTAAGGAGGCAAGGCCTGGGGACACCCGAGATGCCTGGTTAT AATTAACCCAGACATGTGGCTGCCCCCCCCCCCCCAACACCTGCT GCCTCTAAAAATAACCCTGTCCCTGGTGGATCCCCTGCATGCGAA GATCTTCGAACAAGGCTGTGGGGGACTGAGGGCAGGCTGTAACA

Table 1: Exemplary vector genome components for use with the present invention.	
Regulatory Element or Transgene	**Sequence**
	GGCTTGGGGGCCAGGGCTTATACGTGCCTGGGACTCCCAAAGTA TTACTGTTCCATGTTCCCGGCGAAGGGCCAGCTGTCCCCCGCCAG CTAGACTCAGCACTTAGTTTAGGAACCAGTGAGCAAGTCAGCCC TTGGGGCAGCCCATACAAGGCCATGGGGCTGGGCAAGCTGCACG CCTGGGTCCGGGGTGGGCACGGTGCCCGGGCAACGAGCTGAAAG CTCATCTGCTCTCAGGGGCCCCTCCCTGGGGACAGCCCCTCCTGG CTAGTCACACCCTGTAGGCTCCTCTATATAACCCAGGGGCACAG GGGCTGCCCTCATTCTACCACCACCTCCACAGCACAGACAGACA CTCAGGAGCCAGCCAGCC [SEQ ID NO:2]
Chicken β-actin-hybrid promoter	CCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCC AATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGG GCGGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGG GCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGC CAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGC GGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGC GGGAG [SEQ ID NO:3]
SV40 intron	GTAAGTTTAGTCTTTTTTGTCTTTTATTTCAGGTCCCGGATCCGGT GGTGGTGCAAATCAAAGAACTGCTCCTCAGTCGATGTTGCCTTTA CTTCTAG [SEQ ID NO:4]
Micro-dystrophin (μDys) transgene	ATGCTTTGGTGGGAAGAAGTAGAGGACTGTTATGAAAGAGAAGA TGTTCAAAAGAAAACATTCACAAAATGGGTAAATGCACAATTTT CTAAGTTTGGGAAGCAGCATATTGAGAACCTCTTCAGTGACCTA CAGGATGGGAGGCGCCTCCTAGACCTCCTCGAAGGCCTGACAGG GCAAAAACTGCCAAAAGAAAAAGGATCCACAAGAGTTCATGCC CTGAACAATGTCAACAAGGCACTGCGGGTTTTGCAGAACAATAA TGTTGATTTAGTGAATATTGGAAGTACTGACATCGTAGATGGAA ATCATAAACTGACTCTTGGTTTGATTTGGAATATAATCCTCCACT GGCAGGTCAAAAATGTAATGAAAAATATCATGGCTGGATTGCAA CAAACCAACAGTGAAAAGATTCTCCTGAGCTGGGTCCGACAATC AACTCGTAATTATCCACAGGTTAATGTAATCAACTTCACCACCAG CTGGTCTGATGGCCTGGCTTTGAATGCTCTCATCCATAGTCATAG GCCAGACCTATTTGACTGGAATAGTGTGGTTTGCCAGCAGTCAG CCACACAACGACTGGAACATGCATTCAACATCGCCAGATATCAA TTAGGCATAGAGAAACTACTCGATCCTGAAGATGTTGATACCAC CTATCCAGATAAGAAGTCCATCTTAATGTACATCACATCACTCTT CCAAGTTTTGCCTCAACAAGTGAGCATTGAAGCCATCCAGGAAG TGGAAATGTTGCCAAGGCCACCTAAAGTGACTAAAGAAGAACAT TTTCAGTTACATCATCAAATGCACTATTCTCAACAGATCACGGTC AGTCTAGCACAGGGATATGAGAGAACTTCTTCCCCTAAGCCTCG

Table 1: Exemplary vector genome components for use with the present invention.	
Regulatory Element or Transgene	**Sequence**
	ATTCAAGAGCTATGCCTACACACAGGCTGCTTATGTCACCACCTC TGACCCTACACGGAGCCCATTTCCTTCACAGCATTTGGAAGCTCC TGAAGACAAGTCATTTGGCAGTTCATTGATGGAGAGTGAAGTAA ACCTGGACCGTTATCAAACAGCTTTAGAAGAAGTATTATCGTGG CTTCTTTCTGCTGAGGACACATTGCAAGCACAAGGAGAGATTTCT AATGATGTGGAAGTGGTGAAAGACCAGTTTCATACTCATGAGGG GTACATGATGGATTTGACAGCCCATCAGGGCCGGGTTGGTAATA TTCTACAATTGGGAAGTAAGCTGATTGGAACAGGAAAATTATCA GAAGATGAAGAAACTGAAGTACAAGAGCAGATGAATCTCCTAA ATTCAAGATGGGAATGCCTCAGGGTAGCTAGCATGGAAAAACAA AGCAATTTACATAGAGTTTTAATGGATCTCCAGAATCAGAAACT GAAAGAGTTGAATGACTGGCTAACAAAAACAGAAGAAAGAACA AGGAAAATGGAGGAAGAGCCTCTTGGACCTGATCTTGAAGACCT AAAACGCCAAGTACAACAACATAAGGTGCTTCAAGAAGATCTAG AACAAGAACAAGTCAGGGTCAATTCTCTCACTCACATGGTGGTG GTAGTTGATGAATCTAGTGGAGATCACGCAACTGCTGCTTTGGA AGAACAACTTAAGGTATTGGGAGATCGATGGGCAAACATCTGTA GATGGACAGAAGACCGCTGGGTTCTTTTACAAGACATCCTTCTCA AATGGCAACGTCTTACTGAAGAACAGTGCCTTTTTAGTGCATGGC TTTCAGAAAAAGAAGATGCAGTGAACAAGATTCACACAACTGGC TTTAAAGATCAAAATGAAATGTTATCAAGTCTTCAAAAACTGGC CGTTTTAAAAGCGGATCTAGAAAAGAAAAAGCAATCCATGGGCA AACTGTATTCACTCAAACAAGATCTTCTTTCAACACTGAAGAATA AGTCAGTGACCCAGAAGACGGAAGCATGGCTGGATAACTTTGCC CGGTGTTGGGATAATTTAGTCCAAAAACTTGAAAAGAGTACAGC ACAGATTTCACAGGCTGTCACCACCACTCAGCCATCACTAACAC AGACAACTGTAATGGAAACAGTAACTACGGTGACCACAAGGGA ACAGATCCTGGTAAAGCATGCTCAAGAGGAACTTCCACCACCAC CTCCCCAAAAGAAGAGGCAGATTACTGTGGATCTTGAAAGACTC CAGGAACTTCAAGAGGCCACGGATGAGCTGGACCTCAAGCTGCG CCAAGCTGAGGTGATCAAGGGATCCTGGCAGCCCGTGGGCGATC TCCTCATTGACTCTCTCCAAGATCACCTCGAGAAAGTCAAGGCAC TTCGAGGAGAAATTGCGCCTCTGAAAGAGAACGTGAGCCACGTC AATGACCTTGCTCGCCAGCTTACCACTTTGGGCATTCAGCTCTCA CCGTATAACCTCAGCACTCTGGAAGACCTGAACACCAGATGGAA GCTTCTGCAGGTGGCCGTCGAGGACCGAGTCAGGCAGCTGCATG AAGCCCACAGGGACTTTGGTCCAGCATCTCAGCACTTTCTTTCCA CGTCTGTCCAGGGTCCCTGGGAGAGAGCCATCTCGCCAAACAAA GTGCCCTACTATATCAACCACGAGACTCAAACAACTTGCTGGGA CCATCCCAAAATGACAGAGCTCTACCAGTCTTTAGCTGACCTGA ATAATGTCAGATTCTCAGCTTATAGGACTGCCATGAAACTCCGA AGACTGCAGAAGGCCCTTTGCTTGGATCTCTTGAGCCTGTCAGCT GCATGTGATGCCTTGGACCAGCACAACCTCAAGCAAAATGACCA GCCCATGGATATCCTGCAGATTATTAATTGTTTGACCACTATTTA TGACCGCCTGGAGCAAGAGCACAACAATTTGGTCAACGTCCCTC

Table 1: Exemplary vector genome components for use with the present invention.	
Regulatory Element or Transgene	**Sequence**
	TCTGCGTGGATATGTGTCTGAACTGGCTGCTGAATGTTTATGATA CGGGACGAACAGGGAGGATCCGTGTCCTGTCTTTTAAAACTGGC ATCATTTCCCTGTGTAAAGCACATTTGGAAGACAAGTACAGATA CCTTTTCAAGCAAGTGGCAAGTTCAACAGGATTTTGTGACCAGC GCAGGCTGGGCCTCCTTCTGCATGATTCTATCCAAATTCCAAGAC AGTTGGGTGAAGTTGCATCCTTTGGGGGCAGTAACATTGAGCCA AGTGTCCGGAGCTGCTTCCAATTTGCTAATAATAAGCCAGAGAT CGAAGCGGCCCTCTTCCTAGACTGGATGAGACTGGAACCCCAGT CCATGGTGTGGCTGCCCGTCCTGCACAGAGTGGCTGCTGCAGAA ACTGCCAAGCATCAGGCCAAATGTAACATCTGCAAAGAGTGTCC AATCATTGGATTCAGGTACAGGAGTCTAAAGCACTTTAATTATG ACATCTGCCAAAGCTGCTTTTTTTCTGGTCGAGTTGCAAAAGGCC ATAAAATGCACTATCCCATGGTGGAATATTGCACTCCGACTACAT CAGGAGAAGATGTTCGAGACTTTGCCAAGGTACTAAAAAACAAA TTTCGAACCAAAAGGTATTTTGCGAAGCATCCCCGAATGGGCTA CCTGCCAGTGCAGACTGTCTTAGAGGGGGACAACATGGAAACTG ACACAATTTAG [SEQ ID NO:5]
SV40-poly(A) tail	AACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGC ATCACAAATTTCACAAATAAAGCATTTTTTTCACTGC [SEQ ID NO:6]
3' AAV2 ITR	AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTC GCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCG GGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAG [SEQ ID NO:7]
SK-CRM4 enhancer	TTCTGAGTTCTCTAAGGTCCCTCACTCCCAACTCAGACCCAAGTC CTGTCAATTCCCATTCAGTGTCTGATCTCCTTCTTCTCACCTTCCC CATCTTCCATTTGACCCAAGCTTCCTGAGCACTCCTCCCATTCCC CTTTTTGGAGTCCTCCTCCTCTCCCAGAACCCAGTAATAAGTGGG CTCCTCCCTGGCCTGGACCCCCATGGTAACCCTATAAGGCGAGG CAGCTGCCATCTGAGGCAGGGAGGGGCTGGTGTGGGAGGCTAAG G [SEQ ID NO:8]
CMV enhancer	CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCA ACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAG TAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTAT TTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATG CCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTG GCAGTACATCTAC

Table 1: Exemplary vector genome components for use with the present invention.	
Regulatory Element or Transgene	**Sequence**
	[SEQ ID NO:9]

[0142] Nucleic acid elements disclosed herein can be ligated together using standard molecular biology techniques to form gene constructs (*see*, for example, "Molecular Cloning: A Laboratory Manual, 2nd Ed." (Sambrook et al., 1989), "Current Protocols in Molecular Biology" (Ausubel et al., 1987)).

[0143] Gene constructs described herein minimally comprise (from 5' to 3'): (i) a promoter, and (ii) a transgene. For example, in some embodiments the promoter is an MHCK7 promoter. In certain embodiments, the transgene comprises a nucleic acid encoding μDys. In further embodiments, said gene constructs can comprise one or more additional regulatory element. In one embodiment, a gene construct comprises from 5' to 3', a promoter, a transgene, and a SV40 poly (A) tail. In another embodiment, a gene construct comprises from 5' to 3', an enhancer, a promoter, a transgene, an SV40 intron and a SV40 poly (A) tail.

[0144] In some embodiments, a gene construct comprises (from 5' to 3'): (i) a promoter, (ii) and SV40 intron, and (iii) a transgene. In certain embodiments, the promoter is an MHCK7 promoter. In certain embodiments, the transgene is a nucleic acid encoding μDys. In further embodiments, said gene constructs can comprise one or more additional regulatory element. In some embodiments, gene constructs described herein comprise a continuous nucleic acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:10. In some embodiments, the gene construct comprises SEQ ID NO:10. In some embodiments, the gene construct consists of SEQ ID NO:10.

Gene Construct 1 (SEQ ID NO:10)
CCTTCAGATTAAAAATAACTGAGGTAAGGGCCTGGGTAGGGGAGGTGGTGTGA
GACGCTCCTGTCTCTCCTCTATCTGCCCATCGGCCCTTTGGGGAGGAGGAATGT
GCCCAAGGACTAAAAAAAGGCCATGGAGCCAGAGGGGCGAGGGCAACAGACC
TTTCATGGGCAAACCTTGGGGCCCTGCTGTCTAGCATGCCCCACTACGGGTCTA
GGCTGCCCATGTAAGGAGGCAAGGCCTGGGGACACCCGAGATGCCTGGTTATA
ATTAACCCAGACATGTGGCTGCCCCCCCCCCCCCAACACCTGCTGCCTCTAAAA

Gene Construct 1 (SEQ ID NO:10)
ATAACCCTGTCCCTGGTGGATCCCCTGCATGCGAAGATCTTCGAACAAGGCTG
TGGGGGACTGAGGGCAGGCTGTAACAGGCTTGGGGGCCAGGGCTTATACGTGC
CTGGGACTCCCAAAGTATTACTGTTCCATGTTCCCGGCGAAGGGCCAGCTGTCC
CCCGCCAGCTAGACTCAGCACTTAGTTTAGGAACCAGTGAGCAAGTCAGCCCT
TGGGGCAGCCCATACAAGGCCATGGGGCTGGGCAAGCTGCACGCCTGGGTCCG
GGGTGGGCACGGTGCCCGGGCAACGAGCTGAAAGCTCATCTGCTCTCAGGGGC
CCCTCCCTGGGGACAGCCCCTCCTGGCTAGTCACACCCTGTAGGCTCCTCTATA
TAACCCAGGGGCACAGGGGCTGCCCTCATTCTACCACCACCTCCACAGCCTTC
AGATTAAAAATAACTGAGGTAAGGGCCTGGGTAGGGGAGGTGGTGTGAGACG
CTCCTGTCTCTCCTCTATCTGCCCATCGGCCCTTTGGGGAGGAGGAATGTGCCC
AAGGACTAAAAAAAGGCCATGGAGCCAGAGGGGCGAGGGCAACAGACCTTTC
ATGGGCAAACCTTGGGGCCCTGCTGTCTAGCATGCCCCACTACGGGTCTAGGC
TGCCCATGTAAGGAGGCAAGGCCTGGGGACACCCGAGATGCCTGGTTATAATT
AACCCAGACATGTGGCTGCCCCCCCCCCCCCAACACCTGCTGCCTCTAAAAAT
AACCCTGTCCCTGGTGGATCCCCTGCATGCGAAGATCTTCGAACAAGGCTGTG
GGGGACTGAGGGCAGGCTGTAACAGGCTTGGGGGCCAGGGCTTATACGTGCCT
GGGACTCCCAAAGTATTACTGTTCCATGTTCCCGGCGAAGGGCCAGCTGTCCC
CCGCCAGCTAGACTCAGCACTTAGTTTAGGAACCAGTGAGCAAGTCAGCCCTT
GGGGCAGCCCATACAAGGCCATGGGGCTGGGCAAGCTGCACGCCTGGGTCCG
GGGTGGGCACGGTGCCCGGGCAACGAGCTGAAAGCTCATCTGCTCTCAGGGGC
CCCTCCCTGGGGACAGCCCCTCCTGGCTAGTCACACCCTGTAGGCTCCTCTATA
TAACCCAGGGGCACAGGGGCTGCCCTCATTCTACCACCACCTCCACAGCACAG
ACAGACACTCAGGAGCCAGCCAGCCGTAAGTTTAGTCTTTTTTGTCTTTTATTT
CAGGTCCCGGATCCGGTGGTGGTGCAAATCAAAGAACTGCTCCTCAGTCGATG
TTGCCTTTACTTCTAGATGCTTTGGTGGGAAGAAGTAGAGGACTGTTATGAAAG
AGAAGATGTTCAAAAGAAAACATTCACAAAATGGGTAAATGCACAATTTTCTA
AGTTTGGGAAGCAGCATATTGAGAACCTCTTCAGTGACCTACAGGATGGGAGG
CGCCTCCTAGACCTCCTCGAAGGCCTGACAGGGCAAAAACTGCCAAAAGAAA
AAGGATCCACAAGAGTTCATGCCCTGAACAATGTCAACAAGGCACTGCGGGTT
TTGCAGAACAATAATGTTGATTTAGTGAATATTGGAAGTACTGACATCGTAGA
TGGAAATCATAAACTGACTCTTGGTTTGATTTGGAATATAATCCTCCACTGGCA
GGTCAAAAATGTAATGAAAAATATCATGGCTGGATTGCAACAAACCAACAGTG

Gene Construct 1 (SEQ ID NO:10)
AAAAGATTCTCCTGAGCTGGGTCCGACAATCAACTCGTAATTATCCACAGGTT
AATGTAATCAACTTCACCACCAGCTGGTCTGATGGCCTGGCTTTGAATGCTCTC
ATCCATAGTCATAGGCCAGACCTATTTGACTGGAATAGTGTGGTTTGCCAGCA
GTCAGCCACACAACGACTGGAACATGCATTCAACATCGCCAGATATCAATTAG
GCATAGAGAAACTACTCGATCCTGAAGATGTTGATACCACCTATCCAGATAAG
AAGTCCATCTTAATGTACATCACATCACTCTTCCAAGTTTTGCCTCAACAAGTG
AGCATTGAAGCCATCCAGGAAGTGGAAATGTTGCCAAGGCCACCTAAAGTGAC
TAAAGAAGAACATTTTCAGTTACATCATCAAATGCACTATTCTCAACAGATCAC
GGTCAGTCTAGCACAGGGATATGAGAGAACTTCTTCCCCTAAGCCTCGATTCA
AGAGCTATGCCTACACACAGGCTGCTTATGTCACCACCTCTGACCCTACACGG
AGCCCATTTCCTTCACAGCATTTGGAAGCTCCTGAAGACAAGTCATTTGGCAGT
TCATTGATGGAGAGTGAAGTAAACCTGGACCGTTATCAAACAGCTTTAGAAGA
AGTATTATCGTGGCTTCTTTCTGCTGAGGACACATTGCAAGCACAAGGAGAGA
TTTCTAATGATGTGGAAGTGGTGAAAGACCAGTTTCATACTCATGAGGGGTAC
ATGATGGATTTGACAGCCCATCAGGGCCGGGTTGGTAATATTCTACAATTGGG
AAGTAAGCTGATTGGAACAGGAAAATTATCAGAAGATGAAGAAACTGAAGTA
CAAGAGCAGATGAATCTCCTAAATTCAAGATGGGAATGCCTCAGGGTAGCTAG
CATGGAAAAACAAAGCAATTTACATAGAGTTTTAATGGATCTCCAGAATCAGA
AACTGAAAGAGTTGAATGACTGGCTAACAAAAACAGAAGAAAGAACAAGGAA
AATGGAGGAAGAGCCTCTTGGACCTGATCTTGAAGACCTAAAACGCCAAGTAC
AACAACATAAGGTGCTTCAAGAAGATCTAGAACAAGAACAAGTCAGGGTCAA
TTCTCTCACTCACATGGTGGTGGTAGTTGATGAATCTAGTGGAGATCACGCAAC
TGCTGCTTTGGAAGAACAACTTAAGGTATTGGGAGATCGATGGGCAAACATCT
GTAGATGGACAGAAGACCGCTGGGTTCTTTTACAAGACATCCTTCTCAAATGG
CAACGTCTTACTGAAGAACAGTGCCTTTTTAGTGCATGGCTTTCAGAAAAAGA
AGATGCAGTGAACAAGATTCACACAACTGGCTTTAAAGATCAAAATGAAATGT
TATCAAGTCTTCAAAAACTGGCCGTTTTAAAAGCGGATCTAGAAAAGAAAAAG
CAATCCATGGGCAAACTGTATTCACTCAAACAAGATCTTCTTTCAACACTGAAG
AATAAGTCAGTGACCCAGAAGACGGAAGCATGGCTGGATAACTTTGCCCGGTG
TTGGGATAATTTAGTCCAAAAACTTGAAAAGAGTACAGCACAGATTTCACAGG
CTGTCACCACCACTCAGCCATCACTAACACAGACAACTGTAATGGAAACAGTA
ACTACGGTGACCACAAGGGAACAGATCCTGGTAAAGCATGCTCAAGAGGAAC

Gene Construct 1 (SEQ ID NO:10)
TTCCACCACCACCTCCCCAAAAGAAGAGGCAGATTACTGTGGATCTTGAAAGA
CTCCAGGAACTTCAAGAGGCCACGGATGAGCTGGACCTCAAGCTGCGCCAAGC
TGAGGTGATCAAGGGATCCTGGCAGCCCGTGGGCGATCTCCTCATTGACTCTCT
CCAAGATCACCTCGAGAAAGTCAAGGCACTTCGAGGAGAAATTGCGCCTCTGA
AAGAGAACGTGAGCCACGTCAATGACCTTGCTCGCCAGCTTACCACTTTGGGC
ATTCAGCTCTCACCGTATAACCTCAGCACTCTGGAAGACCTGAACACCAGATG
GAAGCTTCTGCAGGTGGCCGTCGAGGACCGAGTCAGGCAGCTGCATGAAGCCC
ACAGGGACTTTGGTCCAGCATCTCAGCACTTTCTTTCCACGTCTGTCCAGGGTC
CCTGGGAGAGAGCCATCTCGCCAAACAAAGTGCCCTACTATATCAACCACGAG
ACTCAAACAACTTGCTGGGACCATCCCAAAATGACAGAGCTCTACCAGTCTTT
AGCTGACCTGAATAATGTCAGATTCTCAGCTTATAGGACTGCCATGAAACTCC
GAAGACTGCAGAAGGCCCTTTGCTTGGATCTCTTGAGCCTGTCAGCTGCATGTG
ATGCCTTGGACCAGCACAACCTCAAGCAAAATGACCAGCCCATGGATATCCTG
CAGATTATTAATTGTTTGACCACTATTTATGACCGCCTGGAGCAAGAGCACAAC
AATTTGGTCAACGTCCCTCTCTGCGTGGATATGTGTCTGAACTGGCTGCTGAAT
GTTTATGATACGGGACGAACAGGGAGGATCCGTGTCCTGTCTTTTAAAACTGG
CATCATTTCCCTGTGTAAAGCACATTTGGAAGACAAGTACAGATACCTTTTCAA
GCAAGTGGCAAGTTCAACAGGATTTTGTGACCAGCGCAGGCTGGGCCTCCTTC
TGCATGATTCTATCCAAATTCCAAGACAGTTGGGTGAAGTTGCATCCTTTGGGG
GCAGTAACATTGAGCCAAGTGTCCGGAGCTGCTTCCAATTTGCTAATAATAAG
CCAGAGATCGAAGCGGCCCTCTTCCTAGACTGGATGAGACTGGAACCCCAGTC
CATGGTGTGGCTGCCCGTCCTGCACAGAGTGGCTGCTGCAGAAACTGCCAAGC
ATCAGGCCAAATGTAACATCTGCAAAGAGTGTCCAATCATTGGATTCAGGTAC
AGGAGTCTAAAGCACTTTAATTATGACATCTGCCAAAGCTGCTTTTTTTCTGGT
CGAGTTGCAAAAGGCCATAAAATGCACTATCCCATGGTGGAATATTGCACTCC
GACTACATCAGGAGAAGATGTTCGAGACTTTGCCAAGGTACTAAAAAACAAAT
TTCGAACCAAAAGGTATTTTGCGAAGCATCCCCGAATGGGCTACCTGCCAGTG
CAGACTGTCTTAGAGGGGGACAACATGGAAACTGACACAATTTAGAACTTGTT
TATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAA
ATAAAGCATTTTTTTCACTGC

[0145] In some embodiments, a gene construct comprises (from 5' to 3'): (i) an enhancer, (ii) a promoter, (iii) a transgene, and (iv) a sequence encoding an SV40 poly(A) tail. In certain embodiments, the enhancer is an SK-CRM4 enhancer. In certain embodiments, the promoter is an MHCK7 promoter. In certain embodiments the transgene is a nucleic acid encoding a μDys. In further embodiments, said gene constructs can comprise one or more additional regulatory element. In some embodiments, a gene construct described herein comprises a continuous nucleic acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:11. In some embodiments, the gene construct comprises a nucleic acid sequence of SEQ ID NO:11. In some embodiments, the gene construct consists of a nucleic acid sequence of SEQ ID NO:11.

[0146] In some embodiments, a vector genome described herein comprises a nucleic acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:12. In some embodiments, the gene construct consists of SEQ ID NO:12. In some embodiments, the vector genome comprises a nucleic acid sequence of SEQ ID NO:12. In some embodiments, the vector genome consists of a nucleic acid sequence of SEQ ID NO:12.

Gene Construct 2 (SEQ ID NO:11)
TTCTGAGTTCTCTAAGGTCCCTCACTCCCAACTCAGACCCAAGTCCTGTCAATT CCCATTCAGTGTCTGATCTCCTTCTTCTCACCTTCCCCATCTTCCATTTGACCCA AGCTTCCTGAGCACTCCTCCCATTCCCCTTTTTGGAGTCCTCCTCCTCTCCCAGA ACCCAGTAATAAGTGGGCTCCTCCCTGGCCTGGACCCCCATGGTAACCCTATA AGGCGAGGCAGCTGCCATCTGAGGCAGGGAGGGGCTGGTGTGGGAGGCTAAG GCCTTCAGATTAAAAATAACTGAGGTAAGGGCCTGGGTAGGGGAGGTGGTGTG AGACGCTCCTGTCTCTCCTCTATCTGCCCATCGGCCCTTTGGGGAGGAGGAATG TGCCCAAGGACTAAAAAAAGGCCATGGAGCCAGAGGGGCGAGGGCAACAGAC CTTTCATGGGCAAACCTTGGGGCCCTGCTGTCTAGCATGCCCCACTACGGGTCT AGGCTGCCCATGTAAGGAGGCAAGGCCTGGGGACACCCGAGATGCCTGGTTAT AATTAACCCAGACATGTGGCTGCCCCCCCCCCCCCAACACCTGCTGCCTCTAAA AATAACCCTGTCCCTGGTGGATCCCCTGCATGCGAAGATCTTCGAACAAGGCT GTGGGGGACTGAGGGCAGGCTGTAACAGGCTTGGGGGCCAGGGCTTATACGTG CCTGGGACTCCCAAAGTATTACTGTTCCATGTTCCCGGCGAAGGGCCAGCTGTC

Gene Construct 2 (SEQ ID NO:11)
CCCCGCCAGCTAGACTCAGCACTTAGTTTAGGAACCAGTGAGCAAGTCAGCCC
TTGGGGCAGCCCATACAAGGCCATGGGGCTGGGCAAGCTGCACGCCTGGGTCC
GGGGTGGGCACGGTGCCCGGGCAACGAGCTGAAAGCTCATCTGCTCTCAGGGG
CCCCTCCCTGGGGACAGCCCCTCCTGGCTAGTCACACCCTGTAGGCTCCTCTAT
ATAACCCAGGGGCACAGGGGCTGCCCTCATTCTACCACCACCTCCACAGCACA
GACAGACACTCAGGAGCCAGCCAGCCATGCTTTGGTGGGAAGAAGTAGAGGA
CTGTTATGAAAGAGAAGATGTTCAAAAGAAAACATTCACAAAATGGGTAAATG
CACAATTTTCTAAGTTTGGGAAGCAGCATATTGAGAACCTCTTCAGTGACCTAC
AGGATGGGAGGCGCCTCCTAGACCTCCTCGAAGGCCTGACAGGGCAAAAACT
GCCAAAAGAAAAAGGATCCACAAGAGTTCATGCCCTGAACAATGTCAACAAG
GCACTGCGGGTTTTGCAGAACAATAATGTTGATTTAGTGAATATTGGAAGTACT
GACATCGTAGATGGAAATCATAAACTGACTCTTGGTTTGATTTGGAATATAATC
CTCCACTGGCAGGTCAAAAATGTAATGAAAAATATCATGGCTGGATTGCAACA
AACCAACAGTGAAAAGATTCTCCTGAGCTGGGTCCGACAATCAACTCGTAATT
ATCCACAGGTTAATGTAATCAACTTCACCACCAGCTGGTCTGATGGCCTGGCTT
TGAATGCTCTCATCCATAGTCATAGGCCAGACCTATTTGACTGGAATAGTGTGG
TTTGCCAGCAGTCAGCCACACAACGACTGGAACATGCATTCAACATCGCCAGA
TATCAATTAGGCATAGAGAAACTACTCGATCCTGAAGATGTTGATACCACCTA
TCCAGATAAGAAGTCCATCTTAATGTACATCACATCACTCTTCCAAGTTTTGCC
TCAACAAGTGAGCATTGAAGCCATCCAGGAAGTGGAAATGTTGCCAAGGCCAC
CTAAAGTGACTAAAGAAGAACATTTTCAGTTACATCATCAAATGCACTATTCTC
AACAGATCACGGTCAGTCTAGCACAGGGATATGAGAGAACTTCTTCCCCTAAG
CCTCGATTCAAGAGCTATGCCTACACACAGGCTGCTTATGTCACCACCTCTGAC
CCTACACGGAGCCCATTTCCTTCACAGCATTTGGAAGCTCCTGAAGACAAGTC
ATTTGGCAGTTCATTGATGGAGAGTGAAGTAAACCTGGACCGTTATCAAACAG
CTTTAGAAGAAGTATTATCGTGGCTTCTTTCTGCTGAGGACACATTGCAAGCAC
AAGGAGAGATTTCTAATGATGTGGAAGTGGTGAAAGACCAGTTTCATACTCAT
GAGGGGTACATGATGGATTTGACAGCCCATCAGGGCCGGGTTGGTAATATTCT
ACAATTGGGAAGTAAGCTGATTGGAACAGGAAAATTATCAGAAGATGAAGAA
ACTGAAGTACAAGAGCAGATGAATCTCCTAAATTCAAGATGGGAATGCCTCAG
GGTAGCTAGCATGGAAAAACAAAGCAATTTACATAGAGTTTTAATGGATCTCC
AGAATCAGAAACTGAAAGAGTTGAATGACTGGCTAACAAAAACAGAAGAAAG

Gene Construct 2 (SEQ ID NO:11)
AACAAGGAAAATGGAGGAAGAGCCTCTTGGACCTGATCTTGAAGACCTAAAA
CGCCAAGTACAACAACATAAGGTGCTTCAAGAAGATCTAGAACAAGAACAAG
TCAGGGTCAATTCTCTCACTCACATGGTGGTGGTAGTTGATGAATCTAGTGGAG
ATCACGCAACTGCTGCTTTGGAAGAACAACTTAAGGTATTGGGAGATCGATGG
GCAAACATCTGTAGATGGACAGAAGACCGCTGGGTTCTTTTACAAGACATCCT
TCTCAAATGGCAACGTCTTACTGAAGAACAGTGCCTTTTTAGTGCATGGCTTTC
AGAAAAAGAAGATGCAGTGAACAAGATTCACACAACTGGCTTTAAAGATCAA
AATGAAATGTTATCAAGTCTTCAAAAACTGGCCGTTTTAAAAGCGGATCTAGA
AAAGAAAAAGCAATCCATGGGCAAACTGTATTCACTCAAACAAGATCTTCTTT
CAACACTGAAGAATAAGTCAGTGACCCAGAAGACGGAAGCATGGCTGGATAA
CTTTGCCCGGTGTTGGGATAATTTAGTCCAAAAACTTGAAAAGAGTACAGCAC
AGATTTCACAGGCTGTCACCACCACTCAGCCATCACTAACACAGACAACTGTA
ATGGAAACAGTAACTACGGTGACCACAAGGGAACAGATCCTGGTAAAGCATG
CTCAAGAGGAACTTCCACCACCACCTCCCCAAAAGAAGAGGCAGATTACTGTG
GATCTTGAAAGACTCCAGGAACTTCAAGAGGCCACGGATGAGCTGGACCTCAA
GCTGCGCCAAGCTGAGGTGATCAAGGGATCCTGGCAGCCCGTGGGCGATCTCC
TCATTGACTCTCTCCAAGATCACCTCGAGAAAGTCAAGGCACTTCGAGGAGAA
ATTGCGCCTCTGAAAGAGAACGTGAGCCACGTCAATGACCTTGCTCGCCAGCT
TACCACTTTGGGCATTCAGCTCTCACCGTATAACCTCAGCACTCTGGAAGACCT
GAACACCAGATGGAAGCTTCTGCAGGTGGCCGTCGAGGACCGAGTCAGGCAG
CTGCATGAAGCCCACAGGGACTTTGGTCCAGCATCTCAGCACTTTCTTTCCACG
TCTGTCCAGGGTCCCTGGGAGAGAGCCATCTCGCCAAACAAAGTGCCCTACTA
TATCAACCACGAGACTCAAACAACTTGCTGGGACCATCCCAAAATGACAGAGC
TCTACCAGTCTTTAGCTGACCTGAATAATGTCAGATTCTCAGCTTATAGGACTG
CCATGAAACTCCGAAGACTGCAGAAGGCCCTTTGCTTGGATCTCTTGAGCCTGT
CAGCTGCATGTGATGCCTTGGACCAGCACAACCTCAAGCAAAATGACCAGCCC
ATGGATATCCTGCAGATTATTAATTGTTTGACCACTATTTATGACCGCCTGGAG
CAAGAGCACAACAATTTGGTCAACGTCCCTCTCTGCGTGGATATGTGTCTGAAC
TGGCTGCTGAATGTTTATGATACGGGACGAACAGGGAGGATCCGTGTCCTGTC
TTTTAAAACTGGCATCATTTCCCTGTGTAAAGCACATTTGGAAGACAAGTACAG
ATACCTTTTCAAGCAAGTGGCAAGTTCAACAGGATTTTGTGACCAGCGCAGGC
TGGGCCTCCTTCTGCATGATTCTATCCAAATTCCAAGACAGTTGGGTGAAGTTG

Gene Construct 2 (SEQ ID NO:11)
CATCCTTTGGGGGCAGTAACATTGAGCCAAGTGTCCGGAGCTGCTTCCAATTTG
CTAATAATAAGCCAGAGATCGAAGCGGCCCTCTTCCTAGACTGGATGAGACTG
GAACCCCAGTCCATGGTGTGGCTGCCCGTCCTGCACAGAGTGGCTGCTGCAGA
AACTGCCAAGCATCAGGCCAAATGTAACATCTGCAAAGAGTGTCCAATCATTG
GATTCAGGTACAGGAGTCTAAAGCACTTTAATTATGACATCTGCCAAAGCTGC
TTTTTTTCTGGTCGAGTTGCAAAAGGCCATAAAATGCACTATCCCATGGTGGAA
TATTGCACTCCGACTACATCAGGAGAAGATGTTCGAGACTTTGCCAAGGTACT
AAAAAACAAATTTCGAACCAAAAGGTATTTTGCGAAGCATCCCCGAATGGGCT
ACCTGCCAGTGCAGACTGTCTTAGAGGGGGACAACATGGAAACTGACACAATT
TAGAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACA
AATTTCACAAATAAAGCATTTTTTTCACTGC

Exemplary vector genome sequence of the present invention (SEQ ID NO:12)
CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGC
GACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTG
GCCAACTCCATCACTAGGGGTTCCTTGTAGTTAATGATTAACCCGCCATGCTAC
TTATCTACGTAGCAAGCTAGCGTACCTCGCGAATGCATCTAGATTCGCGACGTT
ACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCC
ATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCA
TTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATC
AAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGG
CCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAG
TACATCTACTCGAGGCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCC
ACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCG
GGGGGGGGGGGGGGGGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGG
GGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCC
GAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGA
AGCGCGCGGCGGGCGGGAGGTAAGTTTAGTCTTTTTGTCTTTTATTTCAGGTCC
CGGATCCGGTGGTGGTGCAAATCAAAGAACTGCTCCTCAGTGGATGTTGCCTTT
ACTTCTAGATGCTTTGGTGGGAAGAAGTAGAGGACTGTTATGAAAGAGAAGAT
GTTCAAAAGAAAACATTCACAAAATGGGTAAATGCACAATTTTCTAAGTTTGG
GAAGCAGCATATTGAGAACCTCTTCAGTGACCTACAGGATGGGAGGCGCCTCC
TAGACCTCCTCGAAGGCCTGACAGGGCAAAAACTGCCAAAAGAAAAAGGATC
CACAAGAGTTCATGCCCTGAACAATGTCAACAAGGCACTGCGGGTTTTGCAGA
ACAATAATGTTGATTTAGTGAATATTGGAAGTACTGACATCGTAGATGGAAAT
CATAAACTGACTCTTGGTTTGATTTGGAATATAATCCTCCACTGGCAGGTCAAA
AATGTAATGAAAAATATCATGGCTGGATTGCAACAAACCAACAGTGAAAAGAT
TCTCCTGAGCTGGGTCCGACAATCAACTCGTAATTATCCACAGGTTAATGTAAT

Exemplary vector genome sequence of the present invention (SEQ ID NO:12)
CAACTTCACCACCAGCTGGTCTGATGGCCTGGCTTTGAATGCTCTCATCCATAG
TCATAGGCCAGACCTATTTGACTGGAATAGTGTGGTTTGCCAGCAGTCAGCCA
CACAACGACTGGAACATGCATTCAACATCGCCAGATATCAATTAGGCATAGAG
AAACTACTCGATCCTGAAGATGTTGATACCACCTATCCAGATAAGAAGTCCAT
CTTAATGTACATCACATCACTCTTCCAAGTTTTGCCTCAACAAGTGAGCATTGA
AGCCATCCAGGAAGTGGAAATGTTGCCAAGGCCACCTAAAGTGACTAAAGAA
GAACATTTTCAGTTACATCATCAAATGCACTATTCTCAACAGATCACGGTCAGT
CTAGCACAGGGATATGAGAGAACTTCTTCCCCTAAGCCTCGATTCAAGAGCTA
TGCCTACACACAGGCTGCTTATGTCACCACCTCTGACCCTACACGGAGCCCATT
TCCTTCACAGCATTTGGAAGCTCCTGAAGACAAGTCATTTGGCAGTTCATTGAT
GGAGAGTGAAGTAAACCTGGACCGTTATCAAACAGCTTTAGAAGAAGTATTAT
CGTGGCTTCTTTCTGCTGAGGACACATTGCAAGCACAAGGAGAGATTTCTAAT
GATGTGGAAGTGGTGAAAGACCAGTTTCATACTCATGAGGGGTACATGATGGA
TTTGACAGCCCATCAGGGCCGGGTTGGTAATATTCTACAATTGGGAAGTAAGC
TGATTGGAACAGGAAAATTATCAGAAGATGAAGAAACTGAAGTACAAGAGCA
GATGAATCTCCTAAATTCAAGATGGGAATGCCTCAGGGTAGCTAGCATGGAAA
AACAAAGCAATTTACATAGAGTTTTAATGGATCTCCAGAATCAGAAACTGAAA
GAGTTGAATGACTGGCTAACAAAAACAGAAGAAAGAACAAGGAAAATGGAGG
AAGAGCCTCTTGGACCTGATCTTGAAGACCTAAAACGCCAAGTACAACAACAT
AAGGTGCTTCAAGAAGATCTAGAACAAGAACAAGTCAGGGTCAATTCTCTCAC
TCACATGGTGGTGGTAGTTGATGAATCTAGTGGAGATCACGCAACTGCTGCTTT
GGAAGAACAACTTAAGGTATTGGGAGATCGATGGGCAAACATCTGTAGATGG
ACAGAAGACCGCTGGGTTCTTTTACAAGACATCCTTCTCAAATGGCAACGTCTT
ACTGAAGAACAGTGCCTTTTTAGTGCATGGCTTTCAGAAAAAGAAGATGCAGT
GAACAAGATTCACACAACTGGCTTTAAAGATCAAAATGAAATGTTATCAAGTC
TTCAAAAACTGGCCGTTTTAAAAGCGGATCTAGAAAAGAAAAAGCAATCCATG
GGCAAACTGTATTCACTCAAACAAGATCTTCTTTCAACACTGAAGAATAAGTC
AGTGACCCAGAAGACGGAAGCATGGCTGGATAACTTTGCCCGGTGTTGGGATA
ATTTAGTCCAAAAACTTGAAAAGAGTACAGCACAGATTTCACAGGCTGTCACC
ACCACTCAGCCATCACTAACACAGACAACTGTAATGGAAACAGTAACTACGGT
GACCACAAGGGAACAGATCCTGGTAAAGCATGCTCAAGAGGAACTTCCACCA
CCACCTCCCCAAAAGAAGAGGCAGATTACTGTGGATCTTGAAAGACTCCAGGA
ACTTCAAGAGGCCACGGATGAGCTGGACCTCAAGCTGCGCCAAGCTGAGGTGA
TCAAGGGATCCTGGCAGCCCGTGGGCGATCTCCTCATTGACTCTCTCCAAGATC
ACCTCGAGAAAGTCAAGGCACTTCGAGGAGAAATTGCGCCTCTGAAAGAGAA
CGTGAGCCACGTCAATGACCTTGCTCGCCAGCTTACCACTTTGGGCATTCAGCT
CTCACCGTATAACCTCAGCACTCTGGAAGACCTGAACACCAGATGGAAGCTTC
TGCAGGTGGCCGTCGAGGACCGAGTCAGGCAGCTGCATGAAGCCCACAGGGA
CTTTGGTCCAGCATCTCAGCACTTTCTTTCCACGTCTGTCCAGGGTCCCTGGGA
GAGAGCCATCTCGCCAAACAAAGTGCCCTACTATATCAACCACGAGACTCAAA
CAACTTGCTGGGACCATCCCAAAATGACAGAGCTCTACCAGTCTTTAGCTGAC
CTGAATAATGTCAGATTCTCAGCTTATAGGACTGCCATGAAACTCCGAAGACT

Exemplary vector genome sequence of the present invention (SEQ ID NO:12)
GCAGAAGGCCCTTTGCTTGGATCTCTTGAGCCTGTCAGCTGCATGTGATGCCTT GGACCAGCACAACCTCAAGCAAAATGACCAGCCCATGGATATCCTGCAGATTA TTAATTGTTTGACCACTATTTATGACCGCCTGGAGCAAGAGCACAACAATTTGG TCAACGTCCCTCTCTGCGTGGATATGTGTCTGAACTGGCTGCTGAATGTTTATG ATACGGGACGAACAGGGAGGATCCGTGTCCTGTCTTTTAAAACTGGCATCATT TCCCTGTGTAAAGCACATTTGGAAGACAAGTACAGATACCTTTTCAAGCAAGT GGCAAGTTCAACAGGATTTTGTGACCAGCGCAGGCTGGGCCTCCTTCTGCATG ATTCTATCCAAATTCCAAGACAGTTGGGTGAAGTTGCATCCTTTGGGGGCAGTA ACATTGAGCCAAGTGTCCGGAGCTGCTTCCAATTTGCTAATAATAAGCCAGAG ATCGAAGCGGCCCTCTTCCTAGACTGGATGAGACTGGAACCCCAGTCCATGGT GTGGCTGCCCGTCCTGCACAGAGTGGCTGCTGCAGAAACTGCCAAGCATCAGG CCAAATGTAACATCTGCAAAGAGTGTCCAATCATTGGATTCAGGTACAGGAGT CTAAAGCACTTTAATTATGACATCTGCCAAAGCTGCTTTTTTTCTGGTCGAGTT GCAAAAGGCCATAAAATGCACTATCCCATGGTGGAATATTGCACTCCGACTAC ATCAGGAGAAGATGTTCGAGACTTTGCCAAGGTACTAAAAAACAAATTTCGAA CCAAAAGGTATTTTGCGAAGCATCCCCGAATGGGCTACCTGCCAGTGCAGACT GTCTTAGAGGGGGACAACATGGAAACTGACACAATTTAGAACTTGTTTATTGC AGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAG CATTTTTTTCACTGCTCGCGAATCGGATCCCCTCGAGTTCTACGTAGATAAGTA GCATGGCGGGTTAATCATTAACTACAAGGAACCCCTAGTGATGGAGTTGGCCA CTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCC CGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAG

AAV Vector Backbones

[0147] In some embodiments, an AAV vector genome of the present invention can be assembled by inserting a gene construct, as described herein, into an appropriate adenovirus plasmid backbone using standard molecular biology techniques (see, for example, Sambrook et al. (1989). "Molecular Cloning: A Laboratory Manual, 2nd Ed."; Ausubel et al. (1987). "Current Protocols in Molecular Biology"). The adenovirus plasmid backbone, in one embodiment, comprises the 5' ITR and 3' ITR sequences described herein. The gene construct is inserted into the adenovirus plasmid backbone between the ITR sequences, downstream of the 5' ITR sequence and upstream of the 3' ITR sequence.

[0148] For example, in one embodiment, an AAV vector genome of the present invention can be assembled by inserting a gene construct comprising the sequence of SEQ ID NO:10 into an adenovirus plasmid backbone comprising the sequence of SEQ ID NO:13.

[0149] In another embodiment, an AAV vector genome of the present invention can be assembled by inserting a gene construct comprising the sequence of SEQ ID NO:11 into an adenovirus plasmid backbone comprising the sequence of SEQ ID NO:13.

[0150] In some embodiments, the adenovirus plasmid backbone of the present invention comprises a nucleic acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:13. In some embodiments, the adenovirus plasmid backbone comprises a nucleic acid sequence of SEQ ID NO:13. In some embodiments, the adenovirus plasmid backbone consists of a nucleic acid sequence of SEQ ID NO:13, provided below.

Exemplary Adenovirus Plasmid Backbone (SEQ ID NO:13)
AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCA
CTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCC
TCAGTGAGCGAGCGAGCGCGCAGCCTTAATTAACCTAAGGAAAATGAAGTGA
AGTTCCTATACTTTCTAGAGAATAGGAACTTCTATAGTGAGTCGAATAAGGGC
GACACAAAATTTATTCTAAATGCATAATAAATACTGATAACATCTTATAGTTTG
TATTATATTTTGTATTATCGTTGACATGTATAATTTTGATATCAAAAACTGATTT
TCCCTTTATTATTTTCGAGATTTATTTTCTTAATTCTCTTTAACAAACTAGAAAT
ATTGTATATACAAAAAATCATAAATAATAGATGAATAGTTTAATTATAGGTGTT
CATCAATCGAAAAAGCAACGTATCTTATTTAAAGTGCGTTGCTTTTTTCTCATT
TATAAGGTTAAATAATTCTCATATATCAAGCAAAGTGACAGGCGCCCTTAAAT
ATTCTGACAAATGCTCTTTCCCTAAACTCCCCCCATAAAAAAACCCGCCGAAG
CGGGTTTTTACGTTATTTGCGGATTAACGATTACTCGTTATCAGAACCGCCCAG
GGGGCCCGAGCTTAACCTTTTTATTTGGGGGAGAGGGAAGTCATGAAAAAACT
AACCTTTGAAATTCGATCTCCAGCACATCAGCAAAACGCTATTCACGCAGTAC
AGCAAATCCTTCCAGACCCAACCAAACCAATCGTAGTAACCATTCAGGAACGC
AACCGCAGCTTAGACCAAAACAGGAAGCTATGGGCCTGCTTAGGTGACGTCTC
TCGTCAGGTTGAATGGCATGGTCGCTGGCTGGATGCAGAAAGCTGGAAGTGTG
TGTTTACCGCAGCATTAAAGCAGCAGGATGTTGTTCCTAACCTTGCCGGGAATG
GCTTTGTGGTAATAGGCCAGTCAACCAGCAGGATGCGTGTAGGCGAATTTGCG
GAGCTATTAGAGCTTATACAGGCATTCGGTACAGAGCGTGGCGTTAAGTGGTC
AGACGAAGCGAGACTGGCTCTGGAGTGGAAAGCGAGATGGGGAGACAGGGCT
GCATGATAAATGTCGTTAGTTTCTCCGGTGGCAGGACGTCAGCATATTTGCTCT

Exemplary Adenovirus Plasmid Backbone (SEQ ID NO:13)
GGCTAATGGAGCAAAAGCGACGGGCAGGTAAAGACGTGCATTACGTTTTCATG
GATACAGGTTGTGAACATCCAATGACATATCGGTTTGTCAGGGAAGTTGTGAA
GTTCTGGGATATACCGCTCACCGTATTGCAGGTTGATATCAACCCGGAGCTTGG
ACAGCCAAATGGTTATACGGTATGGGAACCAAAGGATATTCAGACGCGAATGC
CTGTTCTGAAGCCATTTATCGATATGGTAAAGAAATATGGCACTCCATACGTCG
GCGGCGCGTTCTGCACTGACAGATTAAAACTCGTTCCCTTCACCAAATACTGTG
ATGACCATTTCGGGCGAGGGAATTACACCACGTGGATTGGCATCAGAGCTGAT
GAACCGAAGCGGCTAAAGCCAAAGCCTGGAATCAGATATCTTGCTGAACTGTC
AGACTTTGAGAAGGAAGATATCCTCGCATGGTGGAAGCAACAACCATTCGATT
TGCAAATACCGGAACATCTCGGTAACTGCATATTCTGCATTAAAAAATCAACG
CAAAAAATCGGACTTGCCTGCAAAGATGAGGAGGGATTGCAGCGTGTTTTTAA
TGAGGTCATCACGGGATCCCATGTGCGTGACGGACATCGGGAAACGCCAAAG
GAGATTATGTACCGAGGAAGAATGTCGCTGGACGGTATCGCGAAAATGTATTC
AGAAAATGATTATCAAGCCCTGTATCAGGACATGGTACGAGCTAAAAGATTCG
ATACCGGCTCTTGTTCTGAGTCATGCGAAATATTTGGAGGGCAGCTTGATTTCG
ACTTCGGGAGGGAAGCTGCATGATGCGATGTTATCGGTGCGGTGAATGCAAAG
AAGATAACCGCTTCCGACCAAATCAACCTTACTGGAATCGATGGTGTCTCCGG
TGTGAAAGAACACCAACAGGGGTGTTACCACTACCGCAGGAAAAGGAGGACG
TGTGGCGAGACAGCGACGAAGTATCACCGACATAATCTGCGAAAACTGCAAAT
ACCTTCCAACGAAACGCACCAGAAATAAACCCAAGCCAATCCCAAAAGAATCT
GACGTAAAAACCTTCAACTACACGGCTCACCTGTGGGATATCCGGTGGCTAAG
ACGTCGTGCGAGGAAAACAAGGTGATTGACCAAAATCGAAGTTACGAACAAG
AAAGCGTCGAGCGAGCTTTAACGTGCGCTAACTGCGGTCAGAAGCTGCATGTG
CTGGAAGTTCACGTGTGTGAGCACTGCTGCGCAGAACTGATGAGCGATCCGAA
TAGCTCGATGCACGAGGAAGAAGATGATGGCTAAACCAGCGCGAAGACGATG
TAAAAACGATGAATGCCGGGAATGGTTTCACCCTGCATTCGCTAATCAGTGGT
GGTGCTCTCCAGAGTGTGGAACCAAGATAGCACTCGAACGACGAAGTAAAGA
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Exemplary Adenovirus Plasmid Backbone (SEQ ID NO:13)
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CATGGTTCATCGCGGATGGAGTGAAAGAGATGCGCTATTACGAAAAAATTGAT
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GCCGTGCGGTTGATATTGCCAAAACAGAGCTGTGGGGGAGAGTTGTCGAGAAA
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Exemplary Adenovirus Plasmid Backbone (SEQ ID NO:13)
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GCCGGACTAAGTAGCAATCTCGCTTATATAACGAGCGTGTTTATCGGCTACATC
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CACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAA
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Exemplary Adenovirus Plasmid Backbone (SEQ ID NO:13)
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CCTATTCTCTAGAAAGTATAGGAACTTCTGAAGTGGGGTCGACTTAATTAAGG

Exemplary Adenovirus Plasmid Backbone (SEQ ID NO:13)
CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGC GACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTG GCCAACTCCATCACTAGGGGTTCCT

Adeno-Associated Viruses (AAV) Particle Production

[0151] AAV particles can be produced by any standard method (see, for example, WO 2001/083692; Masic et al. 2014. Molecular Therapy, 22(11):1900-1909; Carter, 1992, Current Opinions in Biotechnology, 1533-539; Muzyczka, 1992, Curr. Topics in Microbial, and Immunol., 158:97- 129); Ratschin et al., Mol. Cell. Biol. 4:2072 (1984); Hermonat et al., Proc. Natl. Acad. Sci. USA, 81:6466 (1984); Tratschin et al., Mol. Cell. Biol. 5:3251 (1985); McLaughlin et al, J. Virol, 62: 1963 (1988); and Lebkowski et al, Mol. Cell. Biol, 7:349 (1988). Samulski et al , J. Virol., 63:3822-3828 (1989); U.S. Patent No. 5,173,414; WO 95/13365; U.S. Patent No. 5,658.776; WO95/13392; WO 96/17947; PCT/US98/18600; WO 97/09441 (PCT/US 96/ 14423); WO 97/08298 (PCT/US96/13872); WO 97/21825 (PCT/US96/20777); WO 97/06243 (PCT/FR96/01064); WO 99/11764; Perrin et al. Vaccine 13: 1244-1250 (1995); Paul et al. Human Gene Therapy 4:609-615 (1993); Clark et al. Gene Therapy 3: 1124- 1132 (1996); U.S. Patent. No. 5,786,211; U.S. Patent No. 5,871,982; and U.S. Patent. No. 6,258,595, herein incorporated by reference in their entireties). For example, in some embodiments, AAV vector genomes described herein can be transformed into *Escherichia coli* to scale-up DNA production, purified using any standard method (for example, a Maxi-Prep K, Thermo Scientific), and verified by restriction digest or sequencing. Purified AAV vector genomes can then be transfected using a standard method (e.g., calcium phosphate transfection, polyethyleneimine, electroporation, and the like) into an appropriate packaging cell line (*e.g.*, HEK293, HeLa, or PerC.6, MRC-5, WI-38, Vera, and FRhL-2 cells) in combination with a plasmid comprising AAV *rep* and AAV *cap* genes, and an AAV helper plasmid. The AAV *rep* and *cap* genes may be from any AAV serotype and may be the same or different from that of the recombinant AAV vector ITRs including, but not limited to, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAVrh.74, AAV8, AAV9, AAV10, AAV11, AAV12, and AAV13. In certain embodiments, AAV particles described herein comprise AAV *rep* and *cap* genes derived from AAV2 and AAV9, respectively.

[0152] In some embodiments, AAV particles described herein can be harvested from packaging cells and purified by methods standard in the art (e.g., Clark et al, Hum. Gene Ther.,

10(6): 1031-1039 (1999); Schenpp and Clark, Methods Mol. Med., 69 427-443 (2002); U.S. Patent No. 6,566,118 and WO 98/09657, incorporated herein in their entirety by reference) such as by cesium chloride ultracentrifugation gradient or column chromatography.

Pharmaceutical Compositions

[0153] The pharmaceutical compositions provided herein are intrathecal pharmaceutical compositions, i.e., are intended for delivery via the intrathecal route. The intrathecal composition comprises an effective amount of an AAV particle encapsidating a μDys transgene, as described herein. In some embodiments, pharmaceutical compositions disclosed herein comprise an AAV particle of the present invention and a pharmaceutically acceptable carrier and, optionally, other medicinal agents, pharmaceutical agents, stabilizing agents, buffers, carriers, adjuvants, diluents, etc. By "pharmaceutically acceptable" it is meant a material that is not toxic or otherwise undesirable, i.e., the material may be administered to a subject without causing any undesirable biological effects. The pharmaceutical composition is an intrathecal pharmaceutical composition. The effective amount of the AAV particle comprises a lower dose of vector genomes compared to an IV AAV pharmaceutical composition comprising the same vector genome components, or the same transgene. For example, in one embodiment, the effective amount of the AAV particle described herein is about 90% or less vector genomes than the effective amount of an IV composition comprising the same AAV particle or the same micro-dystrophin transgene.

[0154] In some embodiments, pharmaceutical compositions provided herein comprise sterile aqueous and non-aqueous injection solutions, which are optionally isotonic with the blood of the subject to whom the pharmaceutical composition is to be delivered. Pharmaceutical compositions can contain antioxidants, buffers, bacteriostats and solutes, which render the composition isotonic with the blood of the intended subject to be administered. Aqueous and non-aqueous sterile suspensions, solutions and emulsions can include suspending agents and thickening agents. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. In some embodiments pharmaceutical compositions comprise pharmaceutically acceptable vehicles and can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Preservatives and other additives may also be present such as, for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like.

[0155] In some embodiments, pharmaceutical compositions can be presented in unit/dose or multi-dose containers, for example, in sealed ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use.

[0156] In some embodiments, pharmaceutical compositions disclosed herein can be alternatively formulated for IV, intramuscular, or intracerebroventricular (ICV) administration.

Methods of Treatment

[0157] One aspect of the present invention relates to a method of treating a dystrophinopathy in a subject in need thereof, comprising intrathecally administering in a single dose, an intrathecal composition comprising an effective amount of an AAV particle described herein. The methods can be employed for preferentially delivering the AAV particle to cardiac and/or skeletal of the subject, for example, to treat a dystrophinopathy such as Duchenne muscular dystrophy (DMD), Becker muscular dystrophy, or DMD-associated dilated cardiomyopathy (DCM). Without wishing to be bound by theory, the AAV particles and methods for delivering the same to subjects in need thereof, to treat a dystrophinopathy, provide a superior benefit to known AAV particles and treatment methods at least because the present invention: (i) allows for significantly lower dosages than IV delivery, in order to achieve substantially the same or a better therapeutic benefit, thereby reducing viral load and toxicity and other side effects; and/or (ii) allows for preferential transgene targeting and expression in cardiac and/or skeletal muscle tissue compared to liver tissue, thereby targeting the transgene to cells of interest to provide a greater therapeutic benefit compared to AAV vectors delivered intravenously; (iii) can benefit greater patient populations compared to IV formulations, because of the lower dosages needed which corresponds to a decreased manufacturing burden.

[0158] In one embodiment, a method of treating a subject in need thereof is provided comprising intrathecally administering to the subject in a single dose, a composition comprising an effective amount of an AAV particle comprising an AAV capsid encapsidating a vector genome comprising a μDys transgene. In a further embodiment, the subject is positioned in the Trendelenburg position prior to the intrathecal administration. In one embodiment, intrathecal administration of the composition is in the absence of a non-ionic, low-osmolar contrast agent. In another embodiment, intrathecal administration is in the presence of a non-ionic, low-osmolar contrast agent.

[0159] The present invention is based in part on the finding that intrathecal administration of an effective amount of an AAV particle described herein allows for μDys transgene expression at higher levels in skeletal and/or cardiac muscle of the subject compared to transgene expression in liver tissue of the subject. For example, in one embodiment, subsequent to administration of the effective amount of the AAV particle, e.g., an AAV9 particle, the μDys transgene is expressed at higher levels in skeletal muscle of the subject, compared to the levels of μDys transgene expression in liver tissue. In another embodiment, subsequent to administration of the effective amount of the AAV particle, e.g., a recombinant AAV9 particle, the μDys transgene is expressed at higher levels in cardiac muscle of the subject, compared to the levels of μDys transgene expression in liver tissue. In yet another embodiment, subsequent to administration of the effective amount of the AAV particle, e.g., an AAV9 particle, the μDys transgene is expressed at higher levels in skeletal and cardiac muscle of the subject, compared to the levels of μDys transgene expression in liver tissue.

[0160] According to the embodiments described herein, transgene expression may refer to gene expression (i.e., by measuring mRNA levels) or expression of the corresponding protein. It will be understood by those of ordinary skill in the art that in order to determine levels of transgene expression in different tissue types, substantially the same amount of tissue, or substantially the same number of cells should be compared for gene expression levels. The higher levels of μDys transgene expression in the skeletal and/or cardiac muscle, in one embodiment, is 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% or at least about 80% higher, compared to the amount of μDys transgene expression in the liver tissue. In a further embodiment, the μDys transgene comprises the nucleic acid sequence set forth in SEQ ID NO:5.

[0161] In one embodiment of the methods described herein, the method of delivering an effective dose of an AAV particle encapsidating a μDys transgene provides greater μDys transgene expression in skeletal and/or cardiac muscle, compared to a substantially identical dose of an AAV particle encapsidating a μDys transgene administered intravenously. In another embodiment, the method of delivering an effective dose of an AAV particle provides greater μDys transgene expression in skeletal and/or cardiac muscle, compared to when an effective amount of an AAV particle encapsidating a μDys transgene is administered intravenously. Transgene expression, in one embodiment, is measured about 1 week, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months,

about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 18 months or about 24 months subsequent to administration of the composition comprising the effective amount of the AAV particle. The transgene, in one embodiment, comprises the nucleic acid sequence set forth in SEQ ID NO:5.

[0162] In another embodiment, an AAV particle encapsidating a μDys transgene of the present invention, when administered intrathecally, provides 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% or at least about 80% greater transgene expression in the skeletal and/or cardiac muscle compared to the transgene expression in the same tissue type when the identical vector genome dose is administered intravenously. The identical vector genome need not contain the same regulatory elements and/or an identical transgene sequence or the same AAV capsid. However, the transgene administered intrathecally and intravenously encode for a μDys polypeptide.

[0163] In yet another embodiment, the ratio of [(skeletal and/or cardiac muscle μDys transgene expression)] / (liver μDys transgene expression)] measured subsequent to administration of the intrathecally administered AAV particle, is greater than the same ratio when the identical dose of an AAV particle encapsidating a μDys transgene, is administered intravenously. Transgene expression, in one embodiment, is measured about 1 week, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 18 months, about 24 months, about 36 months, about 48 months or about 60 months, subsequent to administration of the composition comprising the effective amount of the AAV particle.

[0164] In one embodiment, the effective amount of the AAV particle encapsidating a μDys transgene, when intrathecally administered, provides a greater efficacy or a greater therapeutic benefit, compared to an AAV particle encapsidating a μDys transgene administered intravenously, at the same dose.

[0165] In some embodiments, the intrathecal (IT) vector genome (vg) dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 90%, about 90% or less, about 85%, about 85% or less, about 80%, about 80% or less, about 75%, about 75% or less, about 70%, about 70% or less, about 60%, about 60% or less, about 50%, about 50% or less, about 40%, about 40% or less, about 30%, about 30% or less, about 25%,

about 25% or less, about 10%, or about 10% or less, an intravenous (IV) vg dose of a vector genome encoding a μDys transgene sufficient to provide the same or substantially the same therapeutic response. The IT vector genome comprises a μDys transgene. The IT and IV μDys transgenes need not include the same nucleic acid sequence. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 90% or less than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 75% or less than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 50% or less than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 25% or less than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 10% or less than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 10-40 times less than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. The therapeutic response in one embodiment, is μDys transgene expression in muscle tissue, e.g., cardiac and/or skeletal muscle tissue. In another embodiment, the therapeutic response is an increase of the subject's score from baseline (i.e., prior to treatment) in the NorthStar Ambulatory Assessment (NSAA). In a further embodiment, the transgene encodes a μDys polypeptide.

[0166] In some embodiments, the intrathecal (IT) vector genome (vg) dose sufficient to provide a therapeutic response for one of the treatment methods described herein is lower than an intravenous (IV) vg dose sufficient to provide the same or substantially the same therapeutic response, wherein the IT and IV vector genomes each includes a transgene that encodes for a μDys polypeptide. However, the respective transgenes need not include the same nucleic acid sequence. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 2-fold, about 5-fold, about 10-fold, about 15-fold, about 20-fold, about 25-fold, about 30-fold, about 35-fold, about 40-fold, about

45-fold, about 50-fold, about 55-fold, about 60-fold, about 65-fold, about 70-fold, about 75-fold, about 80-fold, about 85-fold, about 90-fold, about 95-fold, about 100-fold, about 150-fold, about 200-fold, about 250-fold, about 500-fold, or about 1000-fold lower than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 10-fold to about 20-fold, about 10-fold to about 30-fold, about 10-fold to about 40-fold, about 10-fold to about 50-fold, about 10-fold to about 75-fold, about 10-fold to about 100-fold, or about 10-fold to about 1000-fold lower than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 25-fold to about 30-fold, about 25-fold to about 40-fold, about 25-fold to about 50-fold, about 25-fold to about 75-fold, about 25-fold to about 100-fold, about 25-fold to about 500-fold, or about 25-fold to about 1000-fold lower than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 50-fold to about 75-fold, about 50-fold to about 100-fold, about 50-fold to about 250-fold, about 50-fold to about 500-fold, or about 50-fold to about 1000-fold lower than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 100-fold to about 200-fold, about 100-fold to about 250-fold, about 100-fold to about 500-fold, or about 100-fold to about 1000-fold lower than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 10-fold to about 40-fold lower than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 25-fold to about 40-fold lower than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 10-fold lower than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 25-fold lower than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT

vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 40-fold lower than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 50-fold lower than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. In some embodiments, the IT vg dose sufficient to provide a therapeutic response for one of the treatment methods described herein is about 100-fold lower than the IV vg dose sufficient to provide the same or substantially the same therapeutic response. The therapeutic response in one embodiment, is transgene expression in muscle tissue, e.g., cardiac and/or skeletal muscle tissue.

[0167] In some embodiments, an effective dose of an intrathecal (IT) composition comprising the AAV particles encapsidating a μDys transgene described herein is lower than the effective dose of an intravenous (IV) composition comprising an AAV particle encapsidating a μDys transgene. In some embodiments, the effective dose of the IT composition comprising the AAV particle is about 90%, about 90% or less, about 85%, about 85% or less, about 80%, about 80% or less, about 75%, about 75% or less, about 70%, about 70% or less, about 60%, about 60% or less, about 50%, about 50% or less, about 40%, about 40% or less, about 30%, about 30% or less, about 25%, about 25% or less, about 10%, or about 10% or less vector genomes than the effective amount of the IV composition. In some embodiments, the effective amount of the IT composition comprising the AAV particle is about 90% or less vector genomes than the effective amount of the IV composition. In some embodiments, the effective amount of the IT composition comprising the AAV particle is about 75% or less vector genomes than the effective amount of the IV composition. In some embodiments, the effective amount of the IT composition comprising the AAV particle is about 50% or less vector genomes than the effective amount of the IV composition. In some embodiments, the effective amount of the IT composition comprising the AAV particle is about 25% or less vector genomes than the effective amount of the IV composition. In some embodiments, the effective amount of the IT composition comprising the AAV particle is about 10% or less vector genomes than the effective amount of the IV composition.

[0168] In some embodiments, an effective dose of an AAV particle in an IT composition is a lower dose than the effective dose of an AAV particle in an IV composition, wherein each AAV particle encapsidates a μDys transgene. In some embodiments, the effective dose of an AAV particle in an IT composition is about 2-fold, about 5-fold, about 10-fold, about 15-fold,

about 20-fold, about 25-fold, about 30-fold, about 35-fold, about 40-fold, about 45-fold, about 50-fold, about 55-fold, about 60-fold, about 65-fold, about 70-fold, about 75-fold, about 80-fold, about 85-fold, about 90-fold, about 95-fold, about 100-fold, about 150-fold, about 200-fold, about 250-fold, about 500-fold, or about 1000-fold lower dose than the effective dose of the AAV particle in the IV composition. In some embodiments, the effective amount (effective dose) of the IT composition comprising the AAV particle is about 10-fold to about 20-fold, about 10-fold to about 30-fold, about 10-fold to about 40-fold, about 10-fold to about 50-fold, about 10-fold to about 75-fold, about 10-fold to about 100-fold, or about 10-fold to about 1000-fold lower dose than the effective amount (effective dose) of the IV composition comprising the same AAV particle, or an AAV particle encapsidating a transgene encoding the same polypeptide as the transgene encapsidated by the AAV particle in the IT composition. In some embodiments, the effective amount of the intrathecal composition comprising the AAV particle is about 25-fold to about 30-fold, about 25-fold to about 40-fold, about 25-fold to about 50-fold, about 25-fold to about 75-fold, about 25-fold to about 100-fold, about 25-fold to about 500-fold, or about 25-fold to about 1000-fold lower dose than the effective amount of the IV composition comprising the same AAV particle, or an AAV particle encapsidating a transgene encoding the same polypeptide as the transgene encapsidated by the AAV particle in the IT composition. In some embodiments, the effective amount of the intrathecal composition comprising the AAV particle is about 50-fold to about 75-fold, about 50-fold to about 100-fold, about 50-fold to about 250-fold, about 50-fold to about 500-fold, or about 50-fold to about 1000-fold lower dose than the effective amount of the IV composition comprising the same AAV particle, or an AAV particle encapsidating a transgene encoding the same polypeptide as the transgene encapsidated by the AAV particle in the IT composition. In some embodiments, the effective amount of the IT composition comprising the AAV particle is about 100-fold to about 200-fold, about 100-fold to about 250-fold, about 100-fold to about 500-fold, or about 100-fold to about 1000-fold lower dose than the effective amount of the IV composition comprising the same AAV particle. In some embodiments, the effective amount of the IT composition comprising the AAV particle is about 25-fold to about 40-fold lower than the effective amount of the IV composition comprising the same AAV particle, or an AAV particle encapsidating a transgene encoding the same polypeptide as the transgene encapsidated by the AAV particle in the IT composition. In some embodiments, the effective amount of the IT composition comprising the AAV particle is about 10-fold lower dose than the effective amount of the IV composition comprising the same AAV particle, or an AAV particle encapsidating a transgene encoding the same polypeptide as the transgene encapsidated by the

AAV particle in the IT composition. In some embodiments, the effective amount of the IT composition comprising the AAV particle is about 25-fold lower dose than the effective amount of the IV composition comprising the same AAV particle, or an AAV particle encapsidating a transgene encoding the same polypeptide as the transgene encapsidated by the AAV particle in the IT composition. In some embodiments, the effective amount of the IT composition comprising the AAV particle is about 40-fold lower dose than the effective amount of the IV composition comprising the same AAV particle, or an AAV particle encapsidating a transgene encoding the same polypeptide as the transgene encapsidated by the AAV particle in the IT composition. In some embodiments, the effective amount of the IT composition comprising the AAV particle is about 50-fold lower dose than the effective amount of the IV composition comprising the same AAV particle, or an AAV particle encapsidating a transgene encoding the same polypeptide as the transgene encapsidated by the AAV particle in the IT composition. In some embodiments, the effective amount of the IT composition comprising the AAV particle is about 100-fold lower dose than the effective amount of the IV composition comprising the same AAV particle, or an AAV particle encapsidating a transgene encoding the same polypeptide as the transgene encapsidated by the AAV particle in the IT composition. In some embodiments, the transgene is a μDys transgene.

[0169] In one embodiment of the treatment methods provided herein, treating comprises decreasing in serum creatine kinase (CK) levels in a subject compared to the serum CK levels prior to treatment. In some embodiments, the serum CK levels are decreased by about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more compared to serum CK levels prior to treatment. In a further embodiment, serum CK levels are assessed prior to treatment with an AAV particle and at about 12 months, about 18 months, about 24 months, or about 30 months subsequent to administration of the AAV particle. In a further embodiment, the AAV particle comprises an AAV9 capsid encapsidating a μDys transgene.

[0170] In another embodiment of the intrathecal treatment methods provided herein, treating comprises decreasing the number of side effects, or a reducing the severity of one or more side effects in the subject being treated, compared to a subject treated via IV administration of an effective amount of the same AAV particle, or different AAV particle encapsidating a μDys transgene. The AAV particle administered intrathecally, in one embodiment, is an AAV9 particle.

[0171] In some embodiments, an AAV particle of the present invention or a pharmaceutical composition comprising the same can be used to treat a dystrophinopathy including, but not limited to, DMD, Becker muscular dystrophy, and DCM.

[0172] In some embodiments, the AAV particle of the present invention is administered once or multiple times to a subject in need of treatment, e.g., a subject with DMD. In some embodiments, the AAV particle is administered once, twice, three times, four times, five times, six times, seven times, eight times, nine times, ten times, or more to a subject in need of treatment. In a preferred embodiment, an intrathecal composition provided herein comprising the AAV particle is administered once to a subject in need of treatment. In a further embodiment, the AAV particle is an AAV9 particle.

[0173] In one embodiment, an effective amount of an AAV particle comprising an AAV capsid encapsidating a μDys transgene as described herein is used in a method for treating DMD in a subject in need of treatment. In a further embodiment, the subject is intrathecally administered a composition comprising the AAV particle in a single dose. In a further embodiment, the AAV particle is an AAV9 particle. In even a further embodiment, the subject is positioned in the Trendelenburg position prior to the administration.

[0174] In one embodiment of a method for treating DMD, a subject in need of treatment is intrathecally administered in a single dose, a composition comprising an effective amount of an AAV particle comprising an AAV capsid encapsidating a μDys transgene. The μDys transgene, in one embodiment, includes a combination of dystrophin elements set forth in FIG. 14. In one embodiment, the vector genome comprises a nucleic acid sequence set forth in SEQ ID NO:5, 10, 11 or 12.

[0175] In one embodiment of a method for treating DMD, treating comprises increasing the subject's score from baseline (i.e., prior to treatment) in the NorthStar Ambulatory Assessment (NSAA). The NSAA is a 17-item rating scale that is used to measure functional motor abilities in ambulatory DMD subjects. The scale is ordinal with 34 as the maximum score indicating fully independent function. Each activity is graded as either a 0 (unable to achieve independently), 1 (modified method but achieves goal independent of physical assistance from another), or 2 (normal – no obvious modification of activity). See, e.g., Mazonne et al. (2009). Neuromuscular Disorders 19, pp. 458-461 and researchrom.com/masterlist/view/18#form2, the disclosure of each of which is incorporated by reference in its entirety for all purposes. The change from baseline, in one embodiment, is

measured 12 months subsequent to administration of the intrathecal composition. In another embodiment, the change from baseline is measured 18 months subsequent to administration of the intrathecal composition. In another embodiment, the change from baseline is measure 24 months subsequent to administration of the intrathecal composition. In even another embodiment, the subject's increased score from baseline in the NSAA as measured 12 months subsequent to administration is substantially unchanged or increased at 18 months subsequent to administration. In yet even another embodiment, the subject's increased score from baseline in the NSAA as measured 12 months subsequent to administration is substantially unchanged or increased at 24 months subsequent to administration. In yet even another embodiment, the subject's increased score from baseline in the NSAA as measured 12 months subsequent to administration is substantially unchanged or increased at 60 months subsequent to administration.

[0176] Increasing the NSAA score, in one embodiment, comprises increasing the NSAA score by from about 5 to about 25, from about 5 to about 20, from about 5 to about 15 or from about 5 to about 10. In another embodiment, increasing the NSAA score, comprises increasing the NSAA score by from about 2 to about 12 points. In another embodiment, increasing the NSAA score, comprises increasing the NSAA score by from about 2 to about 10 points. In yet another embodiment, increasing the NSAA score, comprises increasing the NSAA score by from about 3 to about 10 points. In even another embodiment, increasing the NSAA score comprises increasing the score by from about 4 to about 10 points. In yet even another embodiment, increasing the NSAA score comprises increasing the score by from about 2 to about 8 points. I n another embodiment, increasing the NSAA score, comprises increasing the NSAA score by from about 2 to about 6 points.

[0177] In one embodiment of a method for treating DMD, a subject in need of treatment is intrathecally administered in a single dose, an effective amount of an AAV vector encapsidating a μDys transgene. The μDys transgene, in one embodiment, includes the combination of dystrophin elements set forth in FIG. 14. In one embodiment, the vector genome comprises the nucleic acid sequence set forth in SEQ ID NO:5, 10, 11 or 12. An effective amount of the AAV vector encapsidating a μDys transgene, in one embodiment, is an amount sufficient to increase the number of meters walked in a 6-minute walk test (6MWT), compared to the number of meters walked prior to treatment.

[0178] The AAV particles of the present invention, or pharmaceutical compositions comprising the same, are administered as a single dose or as divided doses. In some

embodiments, the dose is 1×10^{9} to 1×10^{16} vector genomes (vg), 2.5×10^{13} to 1×10^{15} vg, 5×10^{13} to 1×10^{15} vg, 7.5×10^{13} to 1×10^{15} vg, 1×10^{14} to 1×10^{15} vg, 2.5×10^{14} to 1×10^{15} vg, 5×10^{14} to 1×10^{15} vg, 7.5×10^{14} to 1×10^{15} vg, 1×10^{13} to 7.5×10^{14} vg, 2.5×10^{13} to 7.5×10^{14} vg, 5×10^{13} to 7.5×10^{14} vg, 7.5×10^{13} to 7.5×10^{14} vg, 1×10^{13} to 5.0×10^{14} vg, 2.5×10^{13} to 5.0×10^{14} vg, 5×10^{13} to 5.0×10^{14} vg, 7.5×10^{13} to 5.0×10^{14} vg, 1×10^{13} to 2.5×10^{14} vg, 2.5×10^{13} to 2.5×10^{14} vg, 5×10^{13} to 2.5×10^{14} vg, 7.5×10^{13} to 2.5×10^{14} vg, 1×10^{13} to 1×10^{14} vg, 2.5×10^{13} to 1×10^{14} vg, 5×10^{13} to 1×10^{14} vg, 7.5×10^{13} to 1×10^{14} vg delivered as a single dose or as divided doses. For example, in some embodiments, AAV particles of the present invention or pharmaceutical compositions comprising the same, is administered as a single dose of 2.5×10^{13} vg. In another embodiment, AAV particles of the present invention or pharmaceutical compositions comprising the same, is administered as a single dose of 5×10^{13} vg. In yet another embodiment, AAV particles of the present invention or pharmaceutical compositions comprising the same, is administered as a single dose of 1×10^{14} vg.

[0179] The AAV particles of the present invention, or pharmaceutical compositions comprising the same, are administered as a single intrathecal dose. In some embodiments, doses for intrathecal delivery can be 1×10^{9} to 1×10^{16} vector genomes (vg), 1×10^{10} to 1×10^{16} vg, 1×10^{11} to 1×10^{16} vg, 1×10^{12} to 1×10^{16} vg, 1×10^{13} to 1×10^{16} vg, 1×10^{14} to 1×10^{16} vg, 1×10^{15} to 1×10^{16} vg, 1×10^{9} to 1×10^{15} vg, 1×10^{9} to 1×10^{14} vg, 1×10^{9} to 1 X 10^{13} vg, 1×10^{9} to 1×10^{12} vg, 1×10^{9} to 1×10^{11} vg, 1×10^{9} to 1×10^{10} vg, 1×10^{13} to 1×10^{15} vector vg, 2.5×10^{13} to 1×10^{15} vg, 5×10^{13} to 1×10^{15} vg, 7.5×10^{13} to 1×10^{15} vg, 1×10^{14} to 1×10^{15} vg, 2.5×10^{14} to 1×10^{15} vg, 5×10^{14} to 1×10^{15} vg, 7.5×10^{14} to 1×10^{15} vg, 1×10^{13} to 7.5×10^{14} vg, 2.5×10^{13} to 7.5×10^{14} vg, 5×10^{13} to 7.5×10^{14} vg, 7.5×10^{13} to 7.5×10^{14} vg, 1×10^{13} to 5.0×10^{14} vg, 2.5×10^{13} to 5.0×10^{14} vg, 5×10^{13} to 5.0×10^{14} vg, 7.5×10^{13} to 5.0×10^{14} vg, 1×10^{13} to 2.5×10^{14} vg, 2.5×10^{13} to 2.5×10^{14} vg, 5×10^{13} to 2.5×10^{14} vg, 7.5×10^{13} to 2.5×10^{14} vg, 1×10^{13} to 1×10^{14} vg, 2.5×10^{13} to 1×10^{14} vg, 5×10^{13} to 1×10^{14} vg, 7.5×10^{13} to 1×10^{14} vg delivered as a single or as divided doses. For example, in some embodiments, AAV particles of the present invention or pharmaceutical compositions comprising the same, can be administered as a single intrathecal dose of 2.5×10^{13} vg. In another embodiment, AAV particles of the present invention or pharmaceutical compositions comprising the same, can be administered as a single intrathecal dose of 5×10^{13} vg. In yet another embodiment, AAV particles of the present invention or pharmaceutical compositions comprising the same, can be administered as a single intrathecal dose of 1×10^{14} vg.

[0180] In other embodiments, AAV particles of the present invention or pharmaceutical compositions comprising the same, can be administered as a single or divided

intracerebroventricular doses. In some embodiments, doses for intracerebroventricular delivery can be 1×10^{13} to 1×10^{15} vg, 2.5×10^{13} to 1×10^{15} vg, 5×10^{13} to 1×10^{15} vg, 7.5×10^{13} to 1×10^{15} vg, 1×10^{14} to 1×10^{15} vg, 2.5×10^{14} to 1×10^{15} vg, 5×10^{14} to 1×10^{15} vg, 7.5×10^{14} to 1×10^{15} vg, 1×10^{13} to 7.5×10^{14} vg, 2.5×10^{13} to 7.5×10^{14} vg, 5×10^{13} to 7.5×10^{14} vg, 7.5×10^{13} to 7.5×10^{14} vg, 1×10^{13} to 5.0×10^{14} vg, 2.5×10^{13} to 5.0×10^{14} vg, 5×10^{13} to 5.0×10^{14} vg, 7.5×10^{13} to 5.0×10^{14} vg, 1×10^{13} to 2.5×10^{14} vg, 2.5×10^{13} to 2.5×10^{14} vg, 5×10^{13} to 2.5×10^{14} vg, 7.5×10^{13} to 2.5×10^{14} vg, 1×10^{13} to 1×10^{14} vg, 2.5×10^{13} to 1×10^{14} vg, 5×10^{13} to 1×10^{14} vg, 7.5×10^{13} to 1×10^{14} vg. For example, in some embodiments, AAV particles of the present invention or pharmaceutical compositions comprising the same, can be administered as a single intracerebroventricular dose of 2.5×10^{13} vg. In another embodiment, AAV particles of the present invention or pharmaceutical compositions comprising the same, can be administered as a single intracerebroventricular dose of 5×10^{13} vg. In yet another embodiment, AAV particles of the present invention or pharmaceutical compositions comprising the same, can be administered as a single intracerebroventricular dose of 1×10^{14} vg.

EXAMPLES

[0181] The invention now being generally described, will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and is not intended to limit the invention.

Select Methods

Intracerebroventricular (ICV) injections at postnatal day 1 (p1)

[0182] Perform Intracerebroventricular injection (ICV) of neonatal pups (p1) via the Cerebral Hemisphere. Neonatals are injected fully awake. Ensure needles are marked for an injection depth of 2mm. The injection site is the midpoint between the ear and eye (location is approximately 0.7 -1.0 mm lateral to the sagittal suture and 0.7 -1.0 mm caudal from the neonatal bregma). P0 is designated as the mouse Date of Birth (DoB). Animals may be injected within 36 hours of discovering the litter.

[0183] If an excessive amount of Dosing solution is determined to have leaked out of the injection site, or the injection site was missed entirely, the animal is excluded from the study.

Intracerebroventricular (ICV) injections at postnatal day 28 (p28)

[0184] Hamilton syringe re loaded with desired volume of dosing solution. Standard volume is 8µl per injection site. Animals are removed individually from cage and placed in anesthesia chamber. Once in chamber, the animal is anaesthetized. Tubing is connected from anesthesia machine to stereotaxic device to allow for continued anaesthetization during injection. The respective animal remains in chamber for about two minutes before being removed and placed on the stereotaxic device. Once the animal is on the stereotaxic device, coordinates for injection are set at medial/lateral (M/L): +/- 1.00 mm, anterior/posterior (A/P): -0.5--0.8 mm, dorsal/ventral (D/F): -2.5 mm.

[0185] An iodine swab is applied at incision site on scalp of animal for sterility purposes. Using a scalpel, a small incision is made into the scalp of the animal and the scalp is gently peeled back to expose cranial region. Once needle is in desired location, syringe plunger is slowly pushed down to inject dosing solution into cranial space. The site of injection is monitored during injection and immediately post injection to confirm quality of injection.

Muscle preparation

[0186] When animals reach the appropriate age, they are weighed and anesthetized (ketamine [80 mg/kg], acepromazine [0.5 mg/kg], and xylazine [16 mg/kg]) via intraperitoneal (i.p.) injection. Dissection of tissues is then carried out. Scissors are used to cut the skin at the ankle and then the skin on both lower legs is pulled back to expose the lower and upper leg muscles. The tibialis anterior on one side is dissected as close to its insertion point and weighed to the nearest 0.1mg and then discarded. Then, 4.0 suture is tied at the myotendinous junction of the proximal and distal ends of the extensor digitorum longus (EDL) and then dissected and placed into Ringer's solution (137 mm NaCl, 5 mm KCl, 2 mm $CaCl_2$, 1 mm $MgSO_4$, 1 mm NaH_2PO_4, 24 mm $NaHCO_3$, 11 mm glucose containing 10 mg/liter curare) that is kept at room temperature.

[0187] After dissecting the EDL, the abdomen is opened, and blood is drawn using a 1 cc syringe from the inferior vena cava (about 300-500 µL). The blood sits at room temperature for 25-35 minutes and then is centrifuged at 3,500×g for 10 minutes at 4 °C. After spinning the supernatant, serum is isolated and placed into a microcentrifuge tube and frozen at -80 °C.

[0188] The Achilles tendon is then cut and pulled back to expose the soleus, plantaris and gastrocnemius. The soleus is dissected out and placed in a tube and frozen in liquid nitrogen-cooled isopentane. The plantaris is blunt dissected free of the gastrocnemius and then cut as

proximally as possible and placed in a tube and frozen in liquid nitrogen-cooled isopentane and stored at -80 °C.

[0189] The gastrocnemius is cut as proximally as possible, weighed to the nearest 0.1 mg and placed in a tube and frozen. The tibialis anterior from the contralateral side is dissected free, weighed to the nearest 0.1mg and pinned on cork at resting length. The EDL from the same side will also be dissected free and pinned on the same cork at resting length. The cork is then immersed in liquid nitrogen-cooled isopentane. After about 30 to about 45 seconds, the cork is placed on dry ice, and wrapped in foil and stored at -80 °C. The quadriceps muscle is dissected and placed in a tube and frozen in liquid nitrogen-cooled isopentane and stored at -80 °C. A small piece of liver is dissected and placed in a tube and frozen in liquid nitrogen-cooled isopentane and stored at -80 °C. The diaphragm is dissected, folded in half, and then folded again, and then placed on cork and pinned. Then, the cork is immersed in liquid nitrogen-cooled isopentane. After 30-45 seconds the cork is placed on dry ice, wrapped in foil, and stored at -80 °C.

[0190] The whole heart is dissected, weighed to the nearest 0.1mg and placed in a tube and frozen in liquid nitrogen-cooled isopentane. The tube is capped and placed in liquid nitrogen until storage at -80 °C.

Example 1: Generation of recombinant adeno-associated virus particles

Molecular Cloning of Micro-Dystrophin Gene Constructs

[0191] A micro-dystrophin (μDys) encoding gene construct, referred to herein as INS1201 and formerly known as MTS-001, was synthesized by operably linking an MHCK7 promoter and an SV40 intron to a polynucleotide encoding μDys and an SV40 poly(A) signal (**FIG. 1A**). An alternative μDys encoding gene construct (referred to herein as INS1212 and formerly known as MTS-003) was synthesized by operably linking an SK-CRM4 enhancer with an MHCK7 promoter to a polynucleotide encoding and an SV40 poly(A) signal (**FIG. 1B**). The INS1201 and INS1212 gene constructs were generated in a Puc57 vector backbone. The INS1201 and INS1212 constructs were confirmed by DNA sequencing and the 4542 bp and 4714 constructs, respectively, were isolated by NruI restriction digest and gel-purified for subsequent cloning into an appropriate AAV backbone.

[0192] The isolated INS1201 and INS1212 gene constructs were each independently blunt cloned into a gel-purified pSZ01 vector backbone containing ITR sites and a kanamycin

resistance gene that was linearized/isolated by NruI restriction digest. Following T4 ligation of the INS1212 construct with the pSZ01 vector backbone and INS1212 construct with the pSZ01 vector backbone, DNA was transformed into *E. coli*, grown and purified using a NEB Monarch® Plasmid Miniprep kit. Gene constructs that had undergone successful ligation to produce complete pSZ01-INS1201 or psZ01-INS1212 plasmid clones were identified by HindIII/BsaI restriction digest.

[0193] pSZ01-INS1201 and psZ01-INS1212 clones were scaled-up by bacterial transformation in *E. coli* using a Maxi-Prep Kit (GeneJET Endo-free Plasmid Maxiprep Kit, ThermoScientific). Correct plasmid sequences were re-confirmed by BsaI/HindIII digest (**FIG. 1C**, lanes 1 and 3). Additionally, restriction digest using SmaI (**FIG. 1C**, lanes 2 and 4) was performed as further confirmation of correct incorporation of ITR sites containing INS1201 and INS1212 within the pSZ01 vector backbone.

Transient Transfection and Viral Packaging

[0194] The confirmed pSZ01-INS1201 and psZ01-INS1212 AAV vectors were transiently transfected into HEK293 cells in combination with adenoviral helper plasmid and a chimeric packaging construct that delivers the AAV2 *rep* gene together with the AAV9 *cap* gene, using a standard calcium phosphate transfection method (for example, as described in Vandendriessche *et al.* (2007. J Thromb Haemost 5:16-24), incorporated by reference herein in its entirety). Two days post transfection, AAV particles were harvested and purified using two successive rounds of cesium chloride density gradient ultracentrifugation. 1µl each of purified INS1201-AAV9 (**FIG. 2**, lane 1) and INS1212-AAV9 (**FIG. 2**, lane 2) were titered by comparing to 1×10^{13} vg AAV2 standard (**FIG. 2**, lane 3 (0.5µl), lane 4 (1 µl), lane 5 (2 µl), lane 6 (4 µl)) resolved by SDS-PAGE and silver-stained.

Example 2: Intramuscular Delivery of AAV9 µDys (INS1201-AAV9 and INS1212-AAV9) results in increased µDys expression in MTX mice.

[0195] INS1201-AAV9 and INS1212-AAV9 were intramuscularly injected into the gastrocnemius muscle of *mdx* mice, a common murine model of Duchenne muscular dystrophy (see, for example Rodino-Klapac *et al.* (2013) *Hum Mol Genet.* 22(24):4929-37, incorporated herein by reference). As shown in **FIG. 3A**, 21 days-post intramuscular injection, gastrocnemius muscle injected with 2.7×10^{11} vg of INS1201-AAV9 (iii) or INS1212-AAV9 (iv) exhibited widespread expression of µDys at significantly higher levels than non-injected *mdx* mice (i) and at comparable levels to wildtype C57/Bl mice (ii). **FIG. 3B** similarly shows

high level expression of μDys in *mdx* mice 21 days post intramuscular injection with 2.7×10^{11} vg of INS1212-AAV9.

Example 3: Intracerebroventricular Delivery of AAV9 μDys (INS1201-AAV9 and INS 1212-AAV9) results in increased μDys expression in *MDX* mice.

[0196] INS1201-AAV9 was intracerebroventicularly injected into *mdx* mice on postnatal day 1 (p1) and tissue samples were collected and analyzed for dystrophin. As shown in **FIG. 4A**, 21 days-post intracerebroventricular (ICV) injection with 1.8×10^{11} vg of AAV, INS1201-AAV9 efficiently targeted and resulted in the expression of μDys in gastrocnemius (i), tibialis anterior (ii), quadriceps (iii), gluteus (iv), triceps (v), diaphragm (vi), and heart (vii) muscle cells with little to no expression in the liver (viii) of *mdx* animals. Similarly, as shown in **FIG. 4B**, 21 days-post ICV injection with 9×10^{10} vg of AAV, INS1201-AAV9 efficiently targeted and resulted in the expression of μDys in gastrocnemius (i), tibialis anterior (ii), quadriceps (iii), gluteus (iv), triceps (v), diaphragm (vi), and heart (vii) muscle cells with little to no expression in the liver (viii).

[0197] INS1212-AAV9 was also intracerebroventicularly injected into *mdx* mice on p1 and tissue samples were collected and immunofluorescently stained for dystrophin. As shown in **FIG. 5**, 21 days-post ICV injection with 9×10^{10} vg of AAV, INS1212-AAV9 efficiently targeted and resulted in the expression of μDys in gastrocnemius (i), tibialis anterior (ii), quadriceps (iii), gluteus (iv), triceps (v), diaphragm (vi), and heart (vii) muscle cells with little to no expression in the liver (viii).

[0198] As shown in the hematoxylin and eosin-stained samples of **FIG. 6A**, 80 days post ICV injection with 9×10^{10} vg (ii) and 2.7×10^{11} vg (iii) of INS1201-AAV9, gastrocnemius muscle tissue exhibited restoration of normal tissue architecture and correction of histopathological features of Duchenne muscular dystrophy as compared to wildtype C57/Bl (i) and non-injected *mdx* mouse controls (iv). As shown in **FIG. 6B**, at 80 days post ICV injection with 9×10^{10} vg (ii) and 2.7×10^{11} vg (iii) of INS1201-AAV9, gastrocnemius muscle tissue exhibited levels of μDys comparable to dystrophin levels in wildtype C57/Bl mice (i), and significantly more than dystrophin levels in non-injected *mdx* mice (iv).

[0199] Similarly, as shown in the hematoxylin and eosin-stained samples of **FIG. 7A(ii)**, 80 days post ICV injection with 9×10^{10} vg of INS1212-AAV9, gastrocnemius muscle tissue exhibited restoration of normal tissue architecture and correction of histopathological features of Duchenne muscular dystrophy as compared to wildtype C57/Bl (**FIG. 7A(i)**) and non-

injected *mdx* mouse controls (**FIG. 7A(iii)**). As shown in **FIG. 7B(ii)**, at 80 days post ICV injection with 9×10^{10} vg of INS1212-AAV9, gastrocnemius muscle tissue exhibited levels of μDys comparable to dystrophin levels in wildtype C57/Bl mice (**FIG. 7B(i)**), and significantly more than dystrophin levels in non-injected *mdx* mice (**FIG. 7A(iii)**).

[0200] As shown in **FIG. 8**, gastrocnemius (**FIG. 8A**), triceps (**FIG. 8C**), tibialis anterior (**FIG. 8E**), and diaphragm (**FIG. 8F**) muscle tissue in *mdx* mice 80 days post ICV injection with 9×10^{10} vg or 2.7×10^{11} vg of INS1201-AAV9 exhibited an increase in mean fiber diameter as compared to non-injected *mdx* mice. *Mdx* mice intracerebroventricularly injected with 9×10^{10} vg or 2.7×10^{11} vg of INS1201-AAV9 also exhibited increased frequency of cells having larger diameters (*e.g.*, 25 μm to 60 μm) 80 days post injection in gastrocnemius (**FIG. 8B**), triceps (**FIG. 8D**), tibialis anterior (**FIG. 8F**), and diaphragm (**FIG. 8H**), as compared to non-injected mice.

[0201] Similarly, as shown in **FIG. 9A**, gastrocnemius muscle tissue in *mdx* mice 80 days post ICV injection with 9×10^{10} vg of INS1212-AAV9 exhibited an increase in mean fiber diameter as compared to non-injected *mdx* mice with a concomitant increase in the frequency of cells having larger diameters (*e.g.*, 25 μm to 60 μm) (**FIG. 9B**).

Example 4: Intracerebroventricular Delivery of INS1201-AAV9 results in Increased μDys expression, Improved Muscle Histology and Reduced Fibrosis in *mdx* Mice.

[0202] *Mdx* mice at post-natal day 28 (p28) (with a window of -1 and +7 days such that no animal is less P27 and no animal greater than P35 at time of injection) were administered via intracerebroventricular (ICV) injection one of the following treatments: (i) 9×10^{9} vg of INS1201-AAV9 (n=6); (ii) 9×10^{10} vg of INS1201-AAV9 (n=7); (iii) 2.7×10^{11} vg of INS1201-AAV9 (n=10); (iv) 5.4×10^{11} vg of INS1201-AAV9 (n=7); (v) 1.2×10^{12} vg of INS1201-AAV9 (n=8) or (vi) vehicle control (TFF formulation buffer, n=11). C57/BL1 age-matched mice were used as a wild type (WT) comparator (n=10).

[0203] Tissues were dissected as described above at approximately postnatal day 120.

[0204] INS1201 copies were measured per diploid genome via droplet digital polymerase chain reaction (ddPCR) using primers specific for the INS1201 transgene. DNA copies of INS1201 are provided in **FIG. 15**. RNA transcript copies are provided in **FIG. 16**. TA: tibialis anterior; EDL: extensor digitorum longus; GAS: gastrocnemius; DIA: diaphragm. RPP30: ribonuclease P/MRP Subunit P30.

[0205] As shown in **FIG. 17**, *mdx* mice administered INS1201-AAV9 at all doses tested exhibited an increase in mean EDL fiber diameter, as compared to non-injected *mdx* mice. Consistent with these findings, m*dx* mice intracerebroventricularly injected with INS1201-AAV9 at all doses also exhibited increased frequency of cells having larger diameters (*e.g.*, 25 μm to 60 μm) in EDL muscle, as compared to non-injected mice.

[0206] As shown in **FIG. 18**, *mdx* mice administered INS1201-AAV9 at the four highest doses tested exhibited an increase in mean TA fiber diameter, as compared to non-injected *mdx* mice. Consistent with these findings, m*dx* mice intracerebroventricularly injected with INS1201-AAV9 at the four highest doses tested (9×10^{10} vg; 2.7×10^{11} vg; 5.4×10^{11} vg; 1.2×10^{12} vg) exhibited increased frequency of cells having larger diameters in TA muscle, as compared to non-injected mice.

[0207] **FIG. 19** shows diaphragm muscle sections stained with picrosirius red, post intracerebroventricular (ICV) injection with various doses of INS1201-AAV9. Picrosirius red is used to visualize collagen content. Diaphragm sections obtained from a wildtype C57/Bl mouse and *mdx* mouse administered vehicle are shown for comparison (top panels). As shown in the lower panels of FIG. 19, the *mdx* mice administered INS1201-AAV9 at the five doses tested (9×10^{9} vg; 9×10^{10} vg; 2.7×10^{11} vg; 5.4×10^{11} vg; 1.2×10^{12} vg) exhibited decreased fibrosis, compared to a *mdx* mouse administered vehicle, as measured by collagen content. Percent collagen in diaphragm muscle is also provided in **FIG. 20**.

[0208] **FIG. 21, top,** are microphraphs of EDL sections obtained from an *mdx* mouse at postnatal day 120 (p120) subsequent to intracerebroventricular (ICV) injection at p28 with 5.4×10^{11} vg of INS1201-AAV9 and stained with hematoxylin and eosin (H&E) (far left); laminin/dapi (second from left); dystrophin (second from right); and a merged image (far right). **FIG. 21**, bottom, shows a EDL muscle section obtained from an *mdx* mouse at postnatal day 120 (p120) subsequent to intracerebroventricular (ICV) injection at p28 with vehicle control, and stained with hematoxylin and eosin (H&E) (far left); laminin/dapi (second from left); dystrophin (second from right); and a merged image (far right). The staining shows greater dystrophin expression in the sample obtained from the mouse treated with INS1201-AAV9 compared to the sample taken from the control mouse. Muscle from the INS1201-AAV9 treated mouse also appear healthier, as evident from the H&E and laminin/dapi stains.

Example 5: ICV Delivery of INS-1201 AAV9 results in improved muscle physiology in *mdx* mice.

[0209] *Mdx* mice at post-natal day 1 (p1) were administered via intracerebroventricular (ICV) injection one of the following treatments: (i) 9×10^{9} vg of INS1201-AAV9; (ii) 9×10^{10} vg of INS-1201-AAV9; (iii) 2.7×10^{11} vg of INS1201-AAV9; (iv) vehicle control (TFF formulation buffer). C57/BL10 age-matched mice (WT) were used as a comparator.

[0210] *Mdx* mice at post-natal day 28 (p28) (with a window of -1 and +7 days such that no animal is less P27 and no animal greater than P35 at time of injection) were administered via intracerebroventricular (ICV) injection one of the following treatments: (i) 9×10^{9} vg of INS1201-AAV9; (ii) 9×10^{10} vg of INS1201-AAV9; (iii) 2.7×10^{11} vg of INS1201-AAV9; (iv) 5.4×10^{11} vg of INS1201-AAV9; (v) 1.2×10^{12} vg of INS1201-AAV9 or (vi) vehicle control (TFF formulation buffer). C57/BL1 age-matched mice were used as a wild type (WT) comparator.

[0211] Muscle dissection and preparation was carried out as described above when animals were from 120 to 135 days old.

Muscle Physiology Experimental Design

Muscle mechanics in EDL

[0212] The EDL is mounted in a specialized chamber containing Ringer's solution (137 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mM NaH_2PO_4, 24 mM $NaHCO_3$, 11 mM glucose containing 10 mg/liter curare) at room temperature. The muscle origin is tied to a rigid post, and the insertion is secured to the arm of a dual-mode ergometer (model 300B; Aurora Scientific, ON, Canada), which allows precise control of muscle length.

[0213] Muscle activation is provided via an electrical stimulator using parallel platinum plate electrodes that extend the length of the muscle. Supramaximal stimulation conditions are established for each experiment by single 0.3 ms twitch pulses of increasing voltage, using +50% more than the value where the force plateaued for experimental testing. Optimal muscle length for assessing force characteristics is determined using a series of twitches while incrementally increasing muscle length (10% increments from slack length) and are defined as the length at which supramaximal stimulation produces maximal twitch force. After establishing optimal length, muscle fiber length (L_f) is measured and the muscle is rested for 2 min.

[0214] The muscle is then stimulated with two twitches spaced 60 s apart (0.3 ms pulse duration) to assess twitch characteristics (twitch tension, half-relaxation time, and time-to-peak tension).

[0215] The muscle is stimulated at increasing frequencies (400 ms train duration and 0.3 ms pulse duration; (i) EDL: 1 Hz, 10 Hz, 30 Hz, 50 Hz, 70 Hz, 120 Hz; (ii) soleus: 1 Hz, 5 Hz, 10 Hz, 20 Hz, 40 Hz, 60 Hz, 80 Hz, and 100 Hz) with 120 s intervals between contractions to determine the force–frequency (F-F) relationship.

[0216] Following the F-F testing, optimal length is re-verified by empirically testing maximal twitch force up to ±20% of the length at the end of testing.

Eccentric contractions

[0217] After completing the F-F curve, all stretches or contractions are performed with two-minute intervals, and stimulation is performed at 100Hz with 400ms train duration and 0.3ms pulse duration for isometric and eccentric contractions. Specifically, following a two-minute rest period from the end of the F-F assessment, the passive mechanical properties of the muscle are measured by imposing a 10% L_f stretch at 0.7Lf/s twice with a 500ms hold before returning to the starting muscle length. Then, two isometric contractions are obtained as a measurement of pre-eccentric contraction (EC) maximal isometric force.

[0218] Each muscle then undergoes an EC bout of ten contractions, during which the muscle is stimulated isometrically for the first 200 ms, after which a 15% Lf length change is imposed at a rate of 2 Lf/s. The muscle is then held at this length for 500ms before returning to the starting muscle length. After 10 EC contractions, two isometric contractions are obtained to determine the post-EC maximal isometric force. At the completion of the final isometric measurements, two additional passive stretches are obtained to determine post-EC passive mechanical measurements.

[0219] Following contraction testing, muscles are trimmed of external tendons, blotted dry and weighed to the nearest 0.01mg. Physiological cross-sectional areas of the muscles are calculated as function of fiber length and muscle mass. For all analysis, force is normalized to the muscle physiological cross-sectional area and reported as stress.

[0220] *Mdx* mice receiving ICV injection on postnatal day 1 (p1) of INS1201-AAV9 at all doses tested ($9x10^9$ vg; $9x10^{10}$ vg; $2.7x10^{11}$ vg) exhibited an attenuated decrease in percent contractile force resulting from EC as compared to *mdx* mice treated with vehicle (**FIG. 10A**).

FIG. 10B similarly shows that mice receiving ICV injection of INS1201-AAV9 on p1, at the three doses tested ($9x10^9$ vg; $9x10^{10}$ vg; $2.7x10^{11}$ vg), exhibited improved muscle physiology as indicated by increased percent post-eccentric contraction stress relative to pre-eccentric contraction stress as compared to vehicle treated mice.

[0221] *Mdx* mice receiving ICV injection on postnatal day 28 (p28) of INS1201-AAV9 at three highest doses tested ($2.7x10^{11}$ vg; $5.4x10^{11}$ vg; $1.2x10^{12}$ vg) exhibited an attenuated decrease in percent contractile force resulting from EC as compared to *mdx* mice treated with vehicle (**FIG. 10C**, **10D**).

[0222] *Mdx* mice receiving ICV injection on postnatal day 28 (p28) of INS1201-AAV9 at three highest doses tested ($2.7x10^{11}$ vg; $5.4x10^{11}$ vg; $1.2x10^{12}$ vg) exhibited improved and stabilized muscle function as shown by an increase in peak muscle force following stimulation at various frequencies as compared to *mdx* mice treated with vehicle (**FIG. 10E**, **FIG. 10F**).

Example 6: One-dose Intrathecal Administration of AAV9 Targets Transgene Delivery to Skeletal and Cardiac Muscle Tissue

[0223] AAV9-CBA-GFP was intrathecally administered to cynomolgus macaques who were screened for anti-AAV9 antibodies using a highly specific anti-AAV9 ELISA. Cynomolgus subjects were treated with 2.5×10^{13} vg, 5×10^{13} vg, or 1×10^{14} vg of AAV9-CBA-GFP via a single intrathecal dose and GFP expression was determined by immunohistochemical analysis with NovaRed GFP immunostain and Vector® HRP substrate, RT-PCR, and western blot analysis.

[0224] As shown in **FIG. 11**, cynomolgus macaques receiving a single intrathecal dose of AAV9-CBA-GFP at 2.5×10^{13} vg (iv), 5×10^{13} vg (v), or 1×10^{14} vg (vi), exhibited increased staining for GFP in gastrocnemius (**FIG.11A**), quadriceps (**FIG.11B**), deltoid (**FIG.11C**), triceps (**FIG.11D**), and biceps (**FIG.11E**) muscle tissue as compared to cynomolgus subjects receiving 5×10^{13} vg (ii), or 1×10^{14} vg (iii) AAV9-CBA-GFP administered intravenously. Similarly, cynomolgus macaques receiving a single intrathecal dose of AAV9-CBA-GFP at 2.5×10^{13} vg (iii), 5×10^{13} vg (iv), or 1×10^{14} vg (v), exhibited increased staining for GFP in diaphragm (**FIG. 11F**), tibialis anterior (**FIG.11G**), and heart (**FIG. 11H**) muscle tissue as compared to cynomolgus subjects receiving 5×10^{13} vg (i), or 1×10^{14} vg (ii) AAV9-CBA-GFP administered intravenously. As shown in **FIGs.11A-E** (i), non-injected cynomolgus subjects exhibited little to no GFP staining.

[0225] As shown in **FIG. 11I**, cynomolgus macaques receiving a single intravenous dose of AAV9-CBA-GFP at 5×10^{13} vg (i), or 1×10^{14} vg (ii), exhibited high levels of GFP staining in liver tissue, whereas subjects receiving a single intrathecal dose of AAV9-CBA-GFP at 2.5×10^{13} vg (iii), 5×10^{13} vg (iv), or 1×10^{14} vg (v), exhibited significantly lower GFP staining.

[0226] As shown in **FIG. 12**, immunohistochemical staining for GFP shown in **FIG. 11** corresponded to protein levels as detected by anti-GFP western blot. Cynomolgus macaques receiving a single intrathecal dose of AAV9-CBA-GFP at 2.5×10^{13} vg (**FIG. 12A**), 5×10^{13} vg (**FIG. 12B**), or 1×10^{14} vg (**FIG. 12C**), exhibited increased GFP levels in biceps (1), triceps (2), deltoid (3), quadriceps (4), gastrocnemius (5), tibialis anterior (6), diaphragm (7) and heart (8) muscle tissue, whereas no GFP protein was detected by western blot in the biceps (**FIG. 12D**, 1) or triceps (**FIG. 12D**, 2) of non-injected subjects.

[0227] As shown in **FIG. 13**, GFP protein levels shown in **FIGs. 11** and **12** corresponded to GFP mRNA levels, wherein cynomolgus macaques receiving a single intrathecal dose of AAV9-CBA-GFP exhibited detectable GFP mRNA expression in biceps (1), triceps (2), deltoid (3), tibialis anterior (4), gastrocnemius (5), quadriceps vastus lateralis (6), diaphragm (7), and heart (8) muscle tissue as analyzed by RT-PCR. Minimal GFP mRNA was detected in liver tissue (9) and no GFP mRNA was detectable in biceps (10), triceps (11), deltoid (12) and quadriceps (13) muscle tissue collected from non-injected subjects.

Example 7: Toxicity and Biodistribution Study of INS1201-AAV9 Following Single Dose Administration of Intracerebroventricular Injection in Juvenile C57BL/6J Mice

[0228] **Test System**

- Species: *Mus musculus*
- Strain: C57BL/6J
- Sex: Male
- Age: Approximately 4 weeks on Day 1
- Weight: Commensurate with age
- Number: 192 (+ 60 extra)
- Caging: As per ASC SOPs
- Minimum Acclimation: 5 days

[0229] **Species/Strain, Number and Sex**. Up to 252 male C57BL/6J (strain# 000664) mice, approximately 4 weeks old at study start, are acquired for this study. Only male mice are used in the study, as the intended patient population is male only.

[0230] **Starting Age and Weight Range**. Animals selected for use in this study will be as uniform in age and weight as possible at start of study. Animals will be approximately 3 weeks of age upon delivery and approximately 4 weeks old on day 1 of study.

[0231] Animals will be fed a species-specific diet, fed *ad libitum*. No contaminants are known to be present in the diet at levels that would interfere with the results of this study. Irradiated water will be available *ad libitum* to each animal.

[0232] The test article and control articles used in this study are provided in Table 2 and Table 3, respectively.

Table 2. Test Articles.

Article	**Dose**	**Active Ingredient**	**Concentration**
INS1201-AAV9	1 (Group 2) $8.0x10^{11}$ vg	AAV-micro-dystrophin	$5x10^{13}$ vg/mL
INS1201-AAV9	2 (Group 3) $4.0x10^{11}$ vg	AAV-micro-dystrophin	$5x10^{13}$ vg/mL
INS1201-AAV9	3 (Group 4) $2.0x10^{11}$ vg	AAV-micro-dystrophin	$2.5x10^{13}$ vg/mL

Table 3. Control Article.

Vehicle Control	**Dose**	**Active Ingredient**	**Concentration**
Formulation Buffer	Vehicle (Group 1)	Pluronic F-68	0.006%

[0233] **Experimental Design**

[0234] **Study Design**

[0235] The study consists of three cohorts: animals assigned to cohort 1 will be terminated on Day 85±10 following injection procedure, animals assigned to cohort 2 will be terminated on Day 43±7 following injection procedure and cohort 3 will be terminated on Day 22±3

following injection procedure. The overall study design is presented in Table 4. Extra animals may be dosed and be used to replace any unscheduled deaths of study group animals that occur as a direct result of procedural activity (i.e., injection, restraint, and handling during injection and/or blood collection). Animals that do not survive the test article administration procedure, die or require early termination prior to Day 4 (3 days post-injection) may be replaced. These animals will not be subject to a gross necropsy. Animals that do not meet the inclusion criteria for age may be replaced as necessary. All animal deaths will be reported, regardless of timing of death postinjection. For Groups 1-4 at each given time point, 5 animals will be used for hematology, blood ddPCR and tissue ddPCR; 5 animals will be used for clinical chemistry and histopathology; and 5 animals for coagulation test and histopathology (n=15/group/time point). All Group 5 animals will be euthanized in Week 12 for hematology, clinical chemistry and coagulation (n = 4 for each test). Five (5) animals from Group 5 will be used for histopathology while tissues from the rest of the animals will be collected and saved.

Table 4. Study design.

Group	Dose	Treatment	Blood Collection and Analysis	Tissue Collection and Analysis	No. of Animals/ time point	Time Points
1 (n=45)	Vehicle	Unilateral ICV P28	Hematology + ddPCR	ddPCR	5	Week 3 (Day 22±3) Week 6 (Day 43±7) Week 12 (Day 85±10)
			Clinical chemistry	Histopathology	5	
			coagulation	Histopathology	5	
2 (n=45)	Dose 1 8.0×10^{11} vg	Bilateral ICV P28	Hematology + ddPCR	ddPCR	5	Week 3 (Day 22±3) Week 6 (Day 43±7) Week 12 (Day 85±10)
			Clinical chemistry	Histopathology	5	
			coagulation	Histopathology	5	
3 (n=45)	Dose 2 4.0×10^{11} vg	Unilateral ICV P28	Hematology + ddPCR	ddPCR	5	Week 3 (Day 22±3) Week 6 (Day 43±7) Week 12 (Day 85±10)
			Clinical chemistry	Histopathology	5	
			coagulation	Histopathology	5	
4 (n=45)	Dose 3 2.0×10^{11} vg	Unilateral ICV P28	Hematology + ddPCR	ddPCR	5	Week 3 (Day 22±3) Week 6 (Day 43±7) Week 12 (Day 85±10)
			Clinical chemistry	Histopathology	5	
			coagulation	Histopathology	5	
5 (n=12)	N/A	Naïve Untreated	Hematology	Histopathology (n=5)	4	Week 12 (Day 85±10)
			Clinical chemistry		4	

Table 4. Study design.						
Group	Dose	Treatment	Blood Collection and Analysis	Tissue Collection and Analysis	No. of Animals/ time point	Time Points
			coagulation		4	

[0236] **Cohort 1 - Day 85±10 Termination**. For Cohort 1 of this study, 60 animals designated to groups 1-4 will receive intracerebroventricular (ICV) injections on Day 1, based on their group allocation (Table 1). An additional 12 animals in Cohort 1 are part of Group 5 animals ("Naïve/untreated", Table 1), which will not be injected. At Study Day 85±10, animals in all groups, 1-5 will be terminated, and blood and tissue samples will be collected as described in Table 5.

Table 5. Cohort 1 blood and tissue samples.				
Group	**Treatment & Dose**	**Number of Animals**	**Terminal Procedure Collection**	
			Blood Analysis	**Tissue Analysis**
1	Vehicle	5	Hematology + ddPCR	ddPCR
		5	Clinical Chemistry	Histopathology
		5	Coagulation	
2	Dose 1 8.0×10^{11} vg	5	Hematology + ddPCR	ddPCR
		5	Clinical Chemistry	Histopathology
		5	Coagulation	
3	Dose 2 4.0×10^{11} vg	5	Hematology + ddPCR	ddPCR
		5	Clinical Chemistry	Histopathology
		5	Coagulation	
4	Dose 3 2.0×10^{11} vg	5	Hematology + ddPCR	ddPCR
		5	Clinical Chemistry	Histopathology
		5	Coagulation	
5	Untreated	4	Hematology	Histopathology (n=5)
		4	Clinical Chemistry	
		4	Coagulation	

[0237] **Cohort 2 – Day 43±7 termination**. For Cohort 2, 60 animals will receive ICV injections on Day 1, based on their group allocation (Table 1). At Study Day 43±7, animals will be terminated, and blood and tissue samples will be collected as described in Table 6.

Table 6. Cohort 2 blood and tissue samples.

Group	Treatment & Dose	Number of Animals	Terminal Procedure Collection	
			Blood Analysis	Tissue Analysis
1	Vehicle	5	Hematology + ddPCR	ddPCR
		5	Clinical Chemistry	Histopathology
		5	Coagulation	
2	Dose 1 8.0×10^{11} vg	5	Hematology + ddPCR	ddPCR
		5	Clinical Chemistry	Histopathology
		5	Coagulation	
3	Dose 2 4.0×10^{11} vg	5	Hematology + ddPCR	ddPCR
		5	Clinical Chemistry	Histopathology
		5	Coagulation	
4	Dose 3 2.0×10^{11} vg	5	Hematology + ddPCR	ddPCR
		5	Clinical Chemistry	Histopathology
		5	Coagulation	

[0238] **Cohort 3 - Day 22±3 Termination**. For Cohort 3, 60 animals will receive ICV injections on Day 1, based on their group allocation (Table 1). At Study Day 22±3, animals will be terminated, and blood and tissue samples will be collected as described in Table 7.

Table 7. Cohort 3 blood and tissue samples.

Group	Treatment & Dose	Number of Animals	Terminal Procedure Collection	
			Blood Analysis	Tissue Analysis
1	Vehicle	5	Hematology + ddPCR	ddPCR
		5	Clinical Chemistry	Histopathology
		5	Coagulation	
2	Dose 1 8.0×10^{11} vg	5	Hematology + ddPCR	ddPCR
		5	Clinical Chemistry	Histopathology
		5	Coagulation	
3	Dose 2 4.0×10^{11} vg	5	Hematology + ddPCR	ddPCR
		5	Clinical Chemistry	Histopathology
		5	Coagulation	
4	Dose 3 2.0×10^{11} vg	5	Hematology + ddPCR	ddPCR
		5	Clinical Chemistry	Histopathology
		5	Coagulation	

[0239] **Group Assignment.** Animals will be ordered and assigned to the study groups in three separate cohorts per Tables 5, 6 and 7. Each cohort of animals, including extras, up to 12 animals per group, will be weighed and assigned a numeric rank from 1 to X according to body weight in a decreasing order (the heaviest animal will be assigned rank = 1). The animals will then be assigned sequentially to each study group based on termination cohort per the study design (see Tables 5, 6, 7).

[0240] **Control for Bias**. All attempts will be made to minimize potential study bias, including: (a) inclusion of appropriate control groups; (b) randomized assignment of animals to study groups; and (c) appropriate staggered dosing of animals across groups.

[0241] **Test Article Administration and Study Procedures**

[0242] **Anesthesia and Surgical Preparation**. Animals are anesthetized using an inhalation anesthetic (1-5% isoflurane with 100% oxygen for induction; 1-3% for maintenance during the surgical procedures). Alternatively, animals are anesthetized with a ketamine (up to 80 mg/kg) and xylazine (up to 12 mg/kg) cocktail administered intraperitoneally.

[0243] **Test Article Preparation and Delivery.** All test article preparation and administration will be performed by Sponsor designated personnel. On Day 1, for each cohort (Tables 5-7), the vehicle and test article will be administered to all animals, via stereotaxic injections into ventricle, based on their group assignment. Test and control article will be kept on ice or between 2-8 °C until ready for use.

[0244] **Cohort injection strategy.** Injections for each termination day cohort will take place over 3-4 days. On each individual injection day, approximately 15-30 mice will be injected. In consideration of test article dose formulations, on the first day of each cohort, injections of all mice of Dose 1 will be completed, followed by vehicle injections to a subset of animals in Group 1 as time allows. On the second day of each cohort, injections of all Dose 2 mice will be completed, followed by vehicle injections to the second subset of animals as time allows for the injection team. On the third day of each cohort, injections of all Dose 3 mice will be completed, followed by vehicle injections to the third subset of animals as time allows for the injection team. If not all vehicle-injected mice have been injected across the first 3 days, the remainder of vehicle injections will take place on the fourth day.

[0245] **Treatment**. Treatments will be administered to animals, in accordance with Tables 5, 6 and 7, by direct injection of treatments into the lateral ventricle. Each animal, depending

on dosing group, will receive either 1 unilateral injection, or 2 injections (bilateral/one injection per side) in a single surgical procedure. Surgical procedures will be performed using stereotaxic instrumentation. Injection devices, surgical instruments, drapes and gowns will be sterile where appropriate. Modifications to the procedures as described below may be performed at the discretion of the surgeon. A single dose of meloxicam (1 mg/kg, SC/IM) and/or buprenorphine (0.01-0.05 mg/kg SC) will be administered to help relieve pain from surgery after induction of anesthesia prior to skull incision. With the animal anesthetized, the skin over the cranium will be shaved (as needed) and the animal mounted in a stereotaxic frame, maintaining the animal on a nose cone for anesthesia with the head positioned by the use of ear bars and the incisor bar as applicable. Aseptic techniques will be used for all surgical procedures. The skin is disinfected with betadine solution followed by 70% alcohol wipe, or equivalent.

[0246] An approximately 2 cm incision will be made at the midline over the skull. Electrocautery may be used to achieve hemostasis of any small hemorrhages from the incision site. A Hamilton syringe with a Hamilton 2-inch, 26-gauge (or smaller), 12-degree bevel stainless steel needle will be attached to the Z-axis of the stereotactic device. The following approximate stereotaxic coordinates, based on bregma, will be used to target the lateral ventricle, either unilaterally or bilaterally:

- Injection Coordinates:
    - Anterior-Posterior (AP): -0.5 to -0.8 mm
    - Medio-Lateral (ML): +/- 1.0 mm*
    - When administering unilateral injections, injection is on right side of the brain. When administering bilateral injections, first injection is on the right, second injection is on the left.
    - Dorso-Ventral (DV): -2.5 mm

[0247] The actual locations of the injections may be adjusted by the surgeon as needed. If the coordinates differ from the ones described above, they will be recorded within the surgical records of the raw data.

[0248] Once needle is in desired location, syringe plunger is slowly pushed down to inject dosing solution into cranial space. The site of injection is monitored during the injection and immediately post injection to confirm quality of injection.

[0249] The desired injection volume is 8 µL per injection site. Injections will target the lateral ventricle and will include 1 or 2 total injections. The needle will be left in place for approximately 1 minute after delivering the treatment to prevent reflux. Any injection abnormalities (leakage, backflow, etc.) will be noted on the Injection sheet for the animal being injected. Following completion of injection(s) and needle removal, the incision is closed using VetBond™ surgical glue, and may be reinforced with a suture. Slight alterations in surgical procedure based on real time status of the animal may be made and will not be considered a deviation to the study protocol.

[0250] Post-Operative Care. Following the surgical procedures, animals are maintained on thermal support through recovery and will be placed in lateral recumbence alternating sides as needed. Mice are administered sterile saline subcutaneously (approximately 0.5 to 1 mL). Animals may also be provided their feed and Diet-Gel on the cage floor as supplemental feed during their surgical recovery.

In-Life Observations and Measurements

[0251] **Routine General Health Observations**. Animals will be observed for changes in general appearance, behavior, and signs of illness during the acclimation period by the husbandry staff, and records will be kept on file as part of the facility records. Daily observations will be recorded beginning on Day 1 and throughout the course of the study until their designated termination day. Any animals exhibiting non-normal clinical signs prior to Day 1 will be excluded from the study.

[0252] **Pre-Study Clinical Observations**. All animals will receive detailed examinations for clinical signs prior to Day 1. Clinical signs indicative of poor health, stress, or other abnormalities will be noted and animals may be excluded from the study at the discretion of the Study Director/Attending Veterinarian and Sponsor Representative.

[0253] **Clinical Observations.** All study animals will receive a detailed clinical observation 6-10 days' post-operatively then once weekly thereafter, in conjunction with body weight, until scheduled termination. Observations noted outside of scheduled observations will be entered as unscheduled. Assessments will include, but will not be limited to: assessment of locomotor activity, neurological observations, posture, respiration, hydration status, surgical site, stereotypic behavior, bladder and bowel (stool) observations, as well as overall body condition.

[0254] The absence or presence of findings during the scheduled clinical observations will be recorded. Clinical signs indicative of poor health, stress, and pain will be noted and will be reported to the Attending Veterinarian. A final detailed examination will be conducted by the Attending Veterinarian for any animal euthanized in extremis.

[0255] **Body Weights**. Individual body weights will be recorded for initial group assignment shortly after animal arrival. Baseline body weights will be taken no more than 4 days prior to dosing. Body weights will be performed once weekly (coinciding with clinical observations) until scheduled termination, and a final body weight will be measured prior to scheduled termination. Per the Study Director's discretion, body weights may be measured more frequently if signs of adverse health or weight loss (i.e., greater than or equal to 10% of the previous week's weight) are observed.

[0256] **Scheduled Sacrifice**. Animals that survive until their scheduled termination date, Study Day 22±3, Day 43±7, Day 85±10, will be humanely euthanized, necropsied, and blood and tissue samples will be collected as described below. Animals will be euthanized in accordance with the AVMA Guidelines for Euthanasia of Animals: 2020 Edition.

Sample/Specimen Collection

[0257] **Blood Collection.** Prior to necropsy, animals may be transferred to an anesthesia box and anesthetized using isoflurane (1-2%) for blood collections for hematology and droplet digital polymerase chain reaction (ddPCR), serum chemistry, and coagulation. Appropriate volume of whole blood will be collected using a 25g needle and 1 cc syringe, or similar, from each animal via cardiac puncture, or other appropriate vessel. Blood samples will be collected from all study animals at their scheduled termination day. Blood will be processed per ASC SOPs and sent to QVL for analysis.

[0258] Samples for Hematology and ddPCR

[0259] For hematology, approximately 250-500 µL of whole blood will be placed into vials containing K_2EDTA as the anticoagulant, inverted gently several times to mix, and placed on wet ice until storage in a refrigerator set to maintain 2 °C to 8 °C.

[0260] Hematology

[0261] Collection Volume: Approximately 250-500 µL

[0262] Anticoagulant: K_2EDTA

[0263] Hematology parameters to be analyzed are provided in Table 8.

Table 8. Hematology parameters to be analyzed.	
White Blood Cell (WBC) Count Differential White Blood Cell Count	Differential White Blood Cell Count (automated and/or hand)
Red Blood Cell (RBC) Count	RBC and WBC Morphology
Hemoglobin Concentration (HGB)	Absolute Reticulocyte Count
Hematocrit (HCT)	Absolute Neutrophil Count
Mean Corpuscular Volume (MCV)	Absolute Lymphocyte Count
Mean Corpuscular Hemoglobin (MCH)	Absolute Monocyte Count
Mean Corpuscular Hemoglobin Concentration (MCHC)	Absolute Eosinophil Count
RBC Distribution Width (RDW)	Absolute Basophil Count
Platelet Count (PLT)	Absolute Large unstained cell count
Mean Platelet Volume (MPV)	Reticulocyte Percent
Blood Smear	

[0264] **Biodistribution by Digital Droplet Polymerase Chain Reaction (ddPCR)**. In addition, approximately 250-500 µL of whole blood will be collected for biodistribution by ddPCR analysis. The whole blood will be placed into K_2EDTA as the anticoagulant, inverted gently several times to mix, and placed on dry ice until storage at -80 °C±10 °C.

[0265] **Samples for Clinical Chemistry**. For clinical chemistry, approximately 500-1000 µL of whole blood will be collected into tubes containing no anticoagulant and allowed to clot at room temperature for at least 30 minutes prior to centrifugation. Clotted whole blood will be centrifuged to yield serum at a temperature of 4 °C, at approximately 3000×g for 5 minutes. The serum samples will be separated following centrifugation, frozen on dry ice immediately after collection and stored frozen at -80 °C±10 °C.

[0266] Serum chemistry parameters to be analyzed are provided in Table 9.

Table 9. Serum chemistry parameters to be analyzed.	
Albumin (ALB)	Calcium (CA)
Anion Gap	Cholesterol (CHOL)
Total Protein (TP)	Glucose (GLU)
Alkaline Phosphatase (ALP)	Inorganic Phosphorous (PHOS)

Table 9. Serum chemistry parameters to be analyzed.	
Alanine Aminotransferase (ALT)	Gamma glutamyl transferase (GGT)
Aspartate Aminotransferase (AST)	Bicarbonate (TCO2)
Creatine Kinase (CK)	Sodium (Na)
Total Bilirubin (TBil)	Potassium (K)
Direct Bilirubin (DBil)	Chloride (Cl)
Indirect Bilirubin (IBil)	Globulin (Glob)
Urea Nitrogen (BUN)	ALB/GLOB ratio (A/G)
Creatine (CREAT)	BUN/CRE ratio (B/c)
Triglycerides	

[0267] Samples for Coagulation. The plasma samples will be separated following centrifugation, and stored in a freezer, set to maintain -10 °C to – 30 °C.

[0268] Coagulation. Collection Volume: Approximately 300-800 µL; Anticoagulant: 3.2 % Sodium citrate; Processing: To plasma. Parameters Analyzed: prothrombin time (PT); activated partial thromboplastin time (APTT); fibrinogen.

[0269] Gross Necropsy. The necropsy will consist of a systematic, macroscopic external and internal examination of the animal's general physical condition and tissues (respiratory, cardiovascular, digestive and urogenital systems). This will be performed for all scheduled and unscheduled sacrifices. Any gross lesions will be documented and preserved. Details on tissue collection are described in detail below. Trained and qualified study personnel will conduct gross necropsies and tissue collection.

[0270] Tissue Collection. Tissues to be collected at termination and analyzed by either histopathology or ddPCR are presented in Table 10.

Table 10. Tissues to be collected and analyzed				
Tissue	**Weighed**	**Collected**	**Histopathology (Histology & Microscopic Evaluation)**	**Biodistribution by ddPCR**
Brain	X	X	X	X
Dorsal Root Ganglia (Cervical Level)		X	X	
Dorsal Root Ganglia (Thoracic Level)		X	X	

Table 10. Tissues to be collected and analyzed

Tissue	**Weighed**	**Collected**	**Histopathology (Histology & Microscopic Evaluation)**	**Biodistribution by ddPCR**
Dorsal Root Ganglia (Lumbar Level)		X	X	
Gross lesions/masses		X	X	
Heart	X	X	X	X
Kidney	X	X	X	X
Large intestine, cecum		X	X	
Large intestine, colon		X	X	
Large intestine, rectum		X	X	
Liver	X	X	X	X
Lung with bronchi	X	X	X	X
Lymph node, inguinal		X	X	X
Pancreas		X	X	X
Small intestine, duodenum		X	X	
Small intestine, ileum		X	X	
Small intestine, jejunam		X	X	X
Spinal Cord		X	X	X
Spleen	X	X	X	X
Stomach		X	X	
Testes a, b	X	X	X	X
Epididymides a	X	X	X	X
Thymus	X	X		
Urinary bladder		X	X	
Muscle Tissue				
Rib #6#7 (with intercostal muscle)	X	X	X	X
Tricepts brachii	X	X	X	X
Quadriceps	X	X	X	X
Gastrocnemius	X	X	X	X
Tibialis anterior	X	X	X	X

Table 10. Tissues to be collected and analyzed				
Tissue	**Weighed**	**Collected**	**Histopathology (Histology & Microscopic Evaluation)**	**Biodistribution by ddPCR**
Extensor digitorium	X	X	X	X
Tongue	X	X	X	X

[0271] **Tissue Collection: Histopathology.** Tissues for histopathology will be collected from animals at their designated termination days per Tables 2, 3 and 4. All tissues marked in Table 6 for histopathology (except eyes), including gross lesions/abnormal tissues, will be collected and fixed in 10% neutral buffered formalin (NBF). Eyes will be fixed in Davidson Solution for 24 to 48 hours then transferred to 70% ethanol.

[0272] Tissues will be trimmed, processed, embedded in paraffin, microtomed, stained with H&E and coverslipped. When possible, muscles will be sectioned both transversely and longitudinally. The resulting slides will be microscopically quality checked. All prepared slides will be evaluated by an ACVP veterinary pathologist. A draft pathology report, consisting of tabulated microscopic data and a discussion of noteworthy changes, will be issued. Photomicrographs will be taken and annotated

[0273] ddPCR analysis. Representative samples of tissues listed in Table 10, will be collected and retained for ddPCR analysis. Specimens collected as one large portion will be placed in tubes, snap frozen in liquid nitrogen, placed in a cooler containing dry ice until placed in a freezer set to maintain -60 °C–80 °C.

Example 8: Biodistribution of INS1201-AAV9 Following Intrathecal Delivery in Nonhuman Primates (NHPs)

[0274] **Test System**

- Genus: Macaca
- Species: *fascicularis*
- Sex: Male
- Weight: from about 2-5 kg

[0275] INS1201-AAV9 biodistribution in the periphery and skeletal muscle is measured in response to intrathecal administration.

[0276] All injections are performed intrathecally into the lumbar space of the spinal cord. Twelve (12) total animals are used, as provided in Table 11:

Table 11.

Group	Approx. Weight of NHP	Dose (total vector genomes (vg)	Test Article	Delivery method	n
1	3 kg	2.0×10^{14}	INS1201-AAV9	Intrathecal (IT)	3
2	3 kg	1.0×10^{14}	INS1201-AAV9	IT	3
3	3 kg	5.0×10^{13}	INS1201-AAV9	IT	3
4	3 kg	2.5×10^{13}	INS1201-AAV9	IT	3

[0277] Animals selected for study will be juvenile or adult monkeys between 2 months and 5 years of age weighing approximately 3kg. Animals are pre-screened and negative for AAV9 antibodies. Pre-screening blood collection will take place within 2 months prior to injection procedure. Animals for screening will be sedated and a blood sample collected.

[0278] Selected animals will be brought to the hospital area a few weeks prior infusion. On the day of infusion (d0), the subjects are sedated and a blood sample is collected for chemistry analysis and cell blood count (CBC). The subjects are then injected with a single dose of INS1201--AAV9 according to the dosing table above. All animals less than 9 months old will be housed with their dams. Animals older than 9 months will be housed in small groups.

[0279] The injection is performed by lumbar puncture into the subarachnoid space of the lumbar thecal sac. For intrathecal (IT) injections, subjects are placed in the lateral decubitus position and the posterior midline injection site at ~L4/5 level identified (below the conus of the spinal cord). Under sterile conditions, a spinal needle with stylet is inserted and subarachnoid cannulation is confirmed with the flow of clear CSF from the needle, as well as injection of a small volume of iohexol followed by intra-procedural myelography. Approximately 1ml CSF will be drained, collected and frozen as baseline sample. This is to alleviate the increase in pressure that is created by the subsequent injection of test article. To improve rostral flow distribution of the test article, the subject will then be tilted in the

trendelenberg position (mild head down position). This is a routine procedure when performing CT myelograms in human subjects. For the CSF taps/IT infusions – hypodermic needles (22G ¾ or 1 ½") can also be used for this purpose

[0280] **In-Life Observations**: Treated NHPs will be kept in isolation to reduce exposure to confounding sources of toxicity. Subjects are observed twice daily for activity, relative skin color and general health by the veterinary staff. The animals will be kept for approximately 21 days post injection. Biodistribution and clinical chemistry tests are performed on samples collected at euthanasia.

[0281] The following segments are collected from the spinal cord for biodistribution studies: cervical spinal cord, thoracic spinal cord, lumbar spinal cord, sacral spinal cord, dorsal root ganglion (DRG) root cervical level, DRG root thoracic level, DRG root lumbar level. For each spinal cord segment, two total pieces are collected. One piece is stored in 4% paraformaldehyde (PFA) and one is flash frozen.

[0282] For muscles and organs, four samples of each are collected. Two are placed in 4% PFA and two are flash frozen. Samples of the following muscles are collected: diaphragm, #6/#7 Rib with intercostal muscle and nerve, psoas muscle, deltoid, pectoralis major, biceps brachii, triceps brachii, rectus femoris, vastus medialis, vastus lateralis, gastrocnemius, tibialis anterior, soleus, tongue, masseter, extensor digitorium, rectus abdominus. Samples of the following organs are collected: heart, liver, lung, kidney, spleen.

[0283] Clinical chemistry tests are as follows: AST, ALT, GGT, Alk Phos, Potassium, Sodium, Chloride, Creatinine, Blood urea nitrogen, CBC.

[0284] Brain samples are collected as follows. The cerebellum is separated from the rest of the brain. The cerebellum is cut into four quadrants with right two quadrants stored in 4% PFA and the left two quadrants flash frozen. The brain is split into right and left hemispheres. The brain is then cut into four quadrants (coronal cuts), with the right two quadrants stored in 4% PFA and the left two quadrants flash frozen.

[0285] Six samples are taken from the quadriceps for subsequent muscle biopsy. Three are stored in 4% PFA and three are flash frozen.

[0286] Cerebral spinal fluid (CSF) and serum are also collected for biodistribution studies.

[0287] Biodistribution studies are carried out on the aforementioned samples by droplet digital polymerase chain reaction (ddPCR).

INCORPORATION BY REFERENCE

[0288] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[0289] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

WHAT IS CLAIMED IS:

1. An intrathecal composition comprising an effective amount of an adeno-associated virus (AAV) particle and a pharmaceutically acceptable carrier, wherein the AAV particle comprises a capsid encapsidating a vector genome, wherein the vector genome comprises from 5' to 3':

a 5' inverted terminal repeat (ITR);

a promoter;

an SV40 intron;

a micro-dystrophin (μDys) transgene;

an SV40 poly (A) tail; and

a 3' ITR,

wherein, the effective amount of the AAV particle comprises about 90% or less vector genomes than the effective amount of an AAV particle encapsidating a μDys transgene in an intravenous composition.

2. An intrathecal composition comprising an effective amount of an adeno-associated virus (AAV) particle and a pharmaceutically acceptable carrier, wherein the AAV particle comprises a capsid encapsidating a vector genome, wherein the vector genome comprises from 5' to 3':

a 5' ITR;

an enhancer;

a promoter;

a micro-dystrophin (μDys) transgene;

an SV40 poly (A) tail; and

a 3' ITR,

wherein, the effective amount of the AAV particle comprises about 90% or less vector genomes than the effective amount of an AAV particle encapsidating a μDys transgene in an intravenous composition.

3. The intrathecal composition of claim 2, wherein the enhancer is an SK-CRM4 enhancer.

4. The intrathecal composition of claim 3, wherein the SK-CRM4 enhancer has a sequence comprising SEQ ID NO:8.

5. The intrathecal composition of claim 2, wherein the enhancer is a cytomegalovirus (CMV) enhancer.

6. The intrathecal composition of claim 5, wherein the cytomegalovirus (CMV) enhancer has a sequence comprising SEQ ID NO:9.

7. The intrathecal composition of claim 2, wherein the vector genome further comprises an SV40 intron 5' (upstream) of the micro-dystrophin transgene and 3' (downstream) of the promoter.

8. The intrathecal composition of any one of claims 1-7, wherein the vector genome comprises a micro-dystrophin (μDys) transgene encoding a μDys protein comprising (i) an N-terminal region (NTD) comprising an actin binding site, (ii) a central rod domain comprising from two to four hinge regions and from four to six spectrin repeats, and (iii) a cysteine rich domain.

9. The intrathecal composition of any one of claims 1-7, wherein the vector genome intravenous composition comprises an AAV particle encapsidating a micro-dystrophin (μDys) transgene encoding a μDys protein comprising (i) an N-terminal region (NTD) comprising an actin binding site, (ii) a central rod domain comprising three hinge regions and four spectrin repeats, and (iii) a cysteine rich domain.

10. The intrathecal composition of claim 8 or 9, wherein the spectrin repeats comprise spectrin repeats 16 and 17.

11. The intrathecal composition of any one of claims 8-10, wherein the spectrin repeats comprise spectrin repeats 1 and 24.

12. The intrathecal composition of any one of claims 8-11, wherein the spectrin repeats comprise spectrin repeat 2.

13. The intrathecal composition of any one of claims 8-11, wherein the spectrin repeats comprise spectrin repeat 22.

14. The intrathecal composition of any one of claims 8-13, wherein the spectrin repeats comprise spectrin repeat 23.

15. The intrathecal composition of claim 8, wherein the spectrin repeats comprise spectrin repeats 1, 16, 17, 23 and 24.

16. The intrathecal composition of claim 8, wherein the spectrin repeats comprise spectrin repeats 1, 2, 3 and 24.

17. The intrathecal composition of claim 8, wherein the spectrin repeats comprise spectrin repeats 1, 2, 22, 23 and 24.

18. The intrathecal composition of any one of claims 8-17, wherein the hinge regions comprise dystrophin hinge regions 1 and 4.

19. The intrathecal composition of any one of claims 8-17, wherein the hinge regions comprise hinge regions 1, 3 and 4.

20. The intrathecal composition of any one of claims 1-9, wherein the micro-dystrophin transgene comprises SEQ ID NO:5.

21. The intrathecal composition of any one of claims 1-9, wherein the micro-dystrophin transgene consists of SEQ ID NO:5.

22. The intrathecal composition of any one of claims 1-21, wherein the 5' ITR is an AAV2 ITR.

23. The intrathecal composition of claim 22, wherein the 5' AAV2 ITR has a sequence comprising of SEQ ID NO:1.

24. The intrathecal composition of claim 22 or 23, wherein the 5' AAV2 ITR has a sequence consisting of SEQ ID NO:1.

25. The intrathecal composition of any one of claims 1-24, wherein the 3' ITR is an AAV2 ITR.

26. The intrathecal composition of claim 25, wherein the 3' AAV2 ITR has a sequence comprising of SEQ ID NO:7.

27. The intrathecal composition of claim 25 or 26, wherein the 3' AAV2 ITR has a sequence consisting of SEQ ID NO:7.

28. The intrathecal composition of any one of claims 1-27, wherein the promoter is an MHCK7 promoter.

29. The intrathecal composition of any one of claims 1-27, wherein the promoter is chicken β-actin hybrid promoter.

30. The intrathecal composition of claim 29, wherein the chicken β-actin hybrid promoter comprises the sequence set forth in SEQ ID NO:3.

31. The intrathecal composition of claim 28, wherein the MHCK7 promoter comprises the sequence set forth in SEQ ID NO:2.

32. The intrathecal composition of claim 28, wherein the MHCK7 promoter consists of the sequence set forth in SEQ ID NO:2.

33. The intrathecal composition of any one of claims 1 and 3-32, wherein the SV40 intron comprises the nucleic acid sequence set forth in SEQ ID NO:4.

34. The intrathecal composition of any one of claims 1 and 3-32, wherein the SV40 intron consists of the nucleic acid sequence set forth in SEQ ID NO:4.

35. The intrathecal composition of any one of claims 1-34, wherein the SV40 poly (A) tail comprises the nucleic acid sequence set forth in SEQ ID NO:6.

36. The intrathecal composition of any one of claims 1-35, wherein the SV40 poly (A) tail consists of the nucleic acid sequence set forth in SEQ ID NO:6.

37. The intrathecal composition of any one of claims 1-36, wherein the capsid comprises one or more AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAVrh.74, AAV8, AAV9, AAV10, AAV11, AAV12, or AAV13 capsid proteins.

38. The intrathecal composition of any one of claims 1-36, wherein the AAV particle is an AAV9 particle and the capsid consists of AAV9 capsid proteins.

39. The intrathecal composition of any one of claims 1-36, wherein the AAV particle is an AAV9 particle and the capsid consists of AAV9 capsid proteins.

40. The intrathecal composition of any one of claims 1-36, wherein the capsid comprises one or more AAV9 capsid proteins.

41. The intrathecal composition of claim 40, wherein the one or more AAV9 capsid proteins comprises AAV9 capsid protein VP1.

42. The intrathecal composition of claim 40 or 41, wherein the one or more AAV9 capsid proteins comprise AAV9 capsid protein VP2.

43. The intrathecal composition of any one of claims 40-42, wherein the one or more AAV9 capsid proteins comprise AAV9 capsid protein VP3.

44. The intrathecal composition of any one of claims 1-43, wherein the AAV particle is an AAV9 particle.

45. The intrathecal composition of any one of claims 1-44, wherein the effective amount of the AAV particle in the intrathecal composition comprises about 80% or less vector genomes than the effective amount of the AAV particle in the intravenous composition.

46. The intrathecal composition of any one of claims 1-42, wherein the effective amount of the AAV particle comprises about 70% or less vector genomes than the effective amount of the AAV particle in the intravenous composition.

47. The intrathecal composition of any one of claims 1-42, wherein the effective amount of the AAV particle comprises about 10 to 40 times less vector genomes than the effective amount of the intravenous composition.

48. A method of treating a dystrophinopathy in a subject in need thereof, comprising, intrathecally administering to the subject, in a single dose, the intrathecal composition of any one of claims 1-47.

49. The method of claim 48, wherein the subject is a male subject from about 6 months to about 7 years old.

50. The method of claim 48, wherein the subject is a male subject from about 1 year to about 7 years old.

51. The method of claim 48, wherein the subject is a male subject from about 2 years to about 7 years old.

52. The method of claim 48, wherein the subject is a male subject from about 3 years to about 7 years old.

53. The method of claim 48, wherein the subject is a male subject from about 4 years to about 7 years old.

54. The method of claim 48, wherein the subject is a male subject from about 5 years to about 7 years old.

55. The method of claim 48, wherein the subject is a male subject from about 2 years to about 6 years old.

56. The method of claim 48, wherein the subject is a male subject from about 2 years to about 5 years old.

57. The method of any one of claims 48-56, wherein the dystrophinopathy is Duchenne muscular dystrophy (DMD).

58. The method of any one of claims 48-56, wherein the dystrophinopathy is Becker muscular dystrophy.

59. The method of any one of claims 48-56, wherein the dystrophinopathy is DMD-associated dilated cardiomyopathy (DCM).

60. The method of any one of claims 48-59, wherein the subject is placed in the Trendelenburg position during the treating.

61. The method of any one of claims 48-60, wherein the intrathecal composition is administered in the absence of a non-ionic, low-osmolar contrast agent.

62. The method of any one of claims 45-61, wherein the effective amount of the AAV particle in the intrathecal composition provides a greater therapeutic response than the identical vector genome dose of the same AAV particle administered intravenously.

63. The method of any one of claims 48-62, wherein the effective amount of the AAV particle in the intrathecal composition provides a greater therapeutic response than the identical vector genome dose of the AAV particle in the intravenous composition when administered intravenously.

64. The method of any one of claims 48-63, wherein the intrathecal composition comprises an effective vector genome dose of about 90%, about 90% or less, about 75%, about 75% or less, about 50%, about 50% or less, about 25%, or about 25% or less than the effective vector genome dose of the AAV particle in the intravenous composition.

65. The method of any one of claims 48-63, wherein intrathecal composition comprises an effective vector genome dose of about 90% or less vector genomes than the effective vector genome dose of the AAV particle in the intravenous composition.

66. The method of any one of claims 48-63, wherein intrathecal composition comprises an effective vector genome dose of about 80% or less vector genomes than the effective vector genome dose of the AAV particle in the intravenous composition.

67. The method of any one of claims 48-63, wherein intrathecal composition comprises an effective vector genome dose of about 75% or less vector genomes than the effective vector genome dose of the AAV particle in the intravenous composition.

68. The method of any one of claims 48-63, wherein intrathecal composition comprises an effective vector genome dose of about 50% or less vector genomes than the effective vector genome dose of the AAV particle in the intravenous composition.

69. The method of any one of claims 48-63, wherein intrathecal composition comprises an effective vector genome dose of about 25% or less vector genomes than the effective vector genome dose of the AAV particle in the intravenous composition.

70. The method of any one of claims 48-69, wherein treating comprises increasing the subject's NorthStar Ambulatory Assessment (NSAA) score subsequent to treatment, compared to a baseline NSAA score of the subject.

71. The method of claim 70, wherein increasing the score comprises increasing the score by from about 5 to about 25, from about 5 to about 20, from about 5 to about 15 or from about 5 to about 10.

72. The method of claim 70, wherein increasing the score comprises increasing the score by from about 2 to about 12 points.

73. The method of claim 70, wherein increasing the score comprises increasing the score by from about 2 to about 10 points.

74. The method of claim 70, wherein increasing the score comprises increasing the score by from about 3 to about 10 points.

75. The method of claim 70, wherein increasing the score comprises increasing the score by from about 4 to about 10 points.

76. The method of claim 0, wherein increasing the score comprises increasing the score by from about 2 to about 8 points.

77. The method of claim 70, wherein increasing the score comprises increasing the score by from about 2 to about 6 points.

78. The method of any one of claims 70-77, wherein the baseline NSAA score of the subject is measured prior to the subject undergoing the treatment method.

79. The method of any one of claims 70-78, wherein the NSAA score subsequent to treatment is measured six (6) months after intrathecal administration of the composition.

80. The method of any one of claims 70-78, wherein the NSAA score subsequent to treatment is measured twelve (12) months after intrathecal administration of the composition.

81. The method of any one of claims 70-78, wherein the NSAA score subsequent to treatment is measured eighteen (18) months after intrathecal administration of the composition.

82. The method of any one of claims 70-78, wherein the NSAA score subsequent to treatment is measured twenty four (24) months after intrathecal administration of the composition.

83. The method of one of claims 48-82, wherein treating comprises increasing the number of meters walked by the subject in the six-minute walk test (6MWT) subsequent to treatment, compared to a baseline number of meters walked by the subject in the 6MWT.

84. The method of claim 83, wherein the baseline number of meters walked by the subject in the 6MWT is measured prior to the subject undergoing the treatment method.

85. The method of claim 83 or 84, wherein the six-minute walk test (6MWT) subsequent to treatment is assessed six (6) months after intrathecal administration of the composition.

86. The method of claim 83 or 84, wherein the six-minute walk test (6MWT) subsequent to treatment is assessed twelve (12) months after intrathecal administration of the composition.

87. The method of claim 83 or 84, wherein the six-minute walk test (6MWT) subsequent to treatment is assessed eighteen (18) months after intrathecal administration of the composition.

88. The method of claim 83 or 84, wherein the six-minute walk test (6MWT) subsequent to treatment is measured twenty four (24) months after intrathecal administration of the composition.

89. The method of claim 83 or 84, wherein the six-minute walk test (6MWT) subsequent to treatment is measured thirty six (36) months after intrathecal administration of the composition.

90. The method of claim 83 or 84, wherein the six-minute walk test (6MWT) subsequent to treatment is measured forty eight (48) months after intrathecal administration of the composition.

91. The method of claim 83 or 84, wherein the six-minute walk test (6MWT) subsequent to treatment is measured sixty (60) months after intrathecal administration of the composition.

92. The method of any one of claims 83-91, wherein increasing the number of meters walked by the subject in the 6MWT comprises increasing by from about 5 meters to about 50 meters, about 5 meters to about 45 meters, about 5 meters to about 40 meters, about 5 meters to about 35 meters, about 5 meters to about 30 meters, about 5 meters to about 25 meters, about 5 meters to about 20 meters, about 5 to about 15 meters or about 5 meters to about 10 meters.

93. The method of any one of claims 48-92, wherein the effective amount of the intrathecally administered AAV particle results in a decreased number of side effects, or a reduced severity of one or more side effects, compared to an AAV particle encapsidating a μDys transgene administered intravenously at an effective dose.

94. The method of any one of claims 48-93, wherein the intrathecal composition provides greater μDys transgene expression in skeletal and cardiac muscle, compared to the amount of μDys transgene expression in liver tissue.

95. The method of any one of claims 48-93, wherein the effective amount of the intrathecal composition provides greater μDys transgene expression in skeletal or cardiac muscle, compared to the amount of μDys transgene expression in liver tissue.

96. The method of any one of claims 48-93, wherein the effective amount of the intrathecal composition provides greater μDys transgene expression in skeletal muscle, compared to the amount of μDys transgene expression in liver tissue.

97. The method of any one of claims 48-93, wherein the effective amount of the intrathecal composition provides greater μDys transgene expression in cardiac muscle, compared to the amount of μDys transgene expression in liver tissue.

98. The method of any one of claims 94-97, wherein the greater μDys transgene expression is 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% or at least about 80% greater expression compared to the amount of μDys transgene expression in the liver tissue.

99. The method of any one of claims 48-98, wherein the therapeutic response measured eighteen (18) months subsequent to administration of the intrathecal composition is substantially the same or better than the therapeutic response measured twelve (12) months subsequent to administration of the intrathecal composition.

100. The method of any one of claims 48-98, wherein the therapeutic response measured twenty-four (24) months subsequent to administration of the intrathecal composition is substantially the same or better than the therapeutic response measured twelve (12) months subsequent to administration of the intrathecal composition.

101. A method for preferentially delivering a micro-dystrophin (μDys) transgene to skeletal and/or cardiac muscle of a subject, comprising,

intrathecally administering to a subject in a single dose, an intrathecal composition comprising an effective amount of an adeno-associated virus serotype 9 (AAV9) particle and

a pharmaceutically acceptable carrier, wherein the AAV9 particle comprises an AAV9 capsid encapsidating a vector genome, wherein the vector genome comprises from 5' to 3':

a 5' ITR;

a promoter;

a μDys transgene;

an SV40 poly (A) tail; and

a 3' ITR,

wherein subsequent to administration, the μDys transgene is expressed at higher levels in the skeletal or cardiac muscle of the subject, compared to the transgene expression in liver tissue of the subject.

102. The method of claim 101, wherein subsequent to administration, the μDys transgene is expressed at higher levels in the skeletal muscle of the subject, compared to the μDys transgene expression in liver tissue of the subject.

103. The method of claim 101 or 102, wherein subsequent to administration, the μDys transgene is expressed at higher levels in the cardiac muscle of the subject, compared to the μDys transgene expression in liver tissue of the subject.

104. The method of claim 101, wherein subsequent to administration, the μDys transgene is expressed at higher levels in the skeletal and cardiac muscle of the subject, compared to the μDys transgene expression in liver tissue of the subject.

105. The method of any one of claims 101-104, wherein the higher levels are 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% or at least about 80% higher, compared to the amount of transgene expression in the liver tissue.

106. The method of any one of claims 101-105, wherein the 5' ITR is an AAV2 ITR.

107. The method of claim 106, wherein the 5' AAV2 ITR comprises the sequence of SEQ ID NO:1.

108. The method of claim 106 or 107, wherein the 5' AAV2 ITR has a sequence consisting of SEQ ID NO:1.

109. The method of any one of claims 101-108, wherein the 3' ITR is an AAV2 ITR.

110. The method of claim 109, wherein the 3' AAV2 ITR has a sequence comprising of SEQ ID NO:7.

111. The method of any one of claims 101-110, wherein the promoter is an MHCK7 promoter.

112. The method of claim 111, wherein the MHCK7 promoter has a sequence comprising of SEQ ID NO:2.

113. The method of any one of claims 101-110, wherein the promoter is a chicken β-actin hybrid promoter.

114. The method of claim 113, wherein the chicken β-actin hybrid promoter has a sequence comprising of SEQ ID NO:3.

115. The method of any one of claims 101-114, wherein the vector genome further comprises an SV40 intron 5' (upstream) of the transgene and 3' (downstream) of the promoter.

116. The method of claim 115, wherein the SV40 intron has a sequence comprising of SEQ ID NO:4.

117. The method of claim 115 or 116, wherein the SV40 intron has a sequence consisting of SEQ ID NO:4.

118. The method of any one of claims 101-117, wherein the SV40 poly (A) tail has a sequence comprising of SEQ ID NO:6.

119. The method of any one of claims 101-118, wherein the vector genome further comprises an enhancer is 3'(downstream) of the 5' ITR and 5' (upstream) of the promoter.

120. The method of claim 119, wherein the enhancer is an SK-CRM4 enhancer.

121. The method of claim 120, wherein the SK-CRM4 enhancer has a sequence comprising of SEQ ID NO:8.

122. The method of claim 119, wherein the enhancer is a cytomegalovirus (CMV) enhancer.

123. The method of claim 122, wherein the cytomegalovirus (CMV) enhancer has a sequence comprising SEQ ID NO:9.

124. The method of any one of claims 101-123, wherein the subject is placed in the Trendelenburg position during the administering.

125. The method of any one of claims 101-124, wherein the composition is administered in the absence of a non-ionic, low-osmolar contrast agent.

126. The method of any one of claims 101-125, wherein the dose of the intrathecally administered AAV9 particle provides greater transgene expression in the skeletal and/or cardiac muscle, compared to the identical dose of the same AAV9 particle administered intravenously.

127. The method of any one of claims 101-126, wherein the ratio of [(skeletal and/or cardiac muscle transgene expression)] / (liver transgene expression)] provided by the intrathecally administered AAV9 particle is greater than the same ratio when the identical dose of the same AAV9 particle is administered intravenously.

128. The method of any one of claims 101-127, wherein the transgene is a micro-dystrophin transgene encoding a micro-dystrophin protein comprising (i) an NTD comprising an actin binding site, (ii) a central rod domain comprising from two to four hinge regions and from four to six spectrin repeats, and (iii) a cysteine rich domain.

129. The method of claim 128, wherein the spectrin repeats comprise spectrin repeats 16 and 17.

130. The method of claim 128 or 129, wherein the spectrin repeats comprise spectrin repeats 1 and 24.

131. The method of any one of claims 128-130, wherein the spectrin repeats comprise spectrin repeat 2.

132. The method of any one of claims 128-130, wherein the spectrin repeats comprise spectrin repeat 22.

133. The method of any one of claims 128-130, wherein the spectrin repeats comprise spectrin repeat 23.

134. The method of claim 128, wherein the spectrin repeats comprise spectrin repeats 16 and 17.

135. The method of claim 128, wherein the spectrin repeats comprise spectrin repeats 1, 16, 17, 23 and 24.

136. The method of claim 128, wherein the spectrin repeats comprise spectrin repeats 1, 2, 3 and 24.

137. The method of claim 128, wherein the spectrin repeats comprise spectrin repeats 1, 2, 22, 23 and 24.

138. The method of any one of claims 128-137, wherein the hinge regions comprise dystrophin hinge regions 1 and 4.

139. The method of any one of claims 128-137, wherein the hinge regions comprise hinge regions 1, 3 and 4.

140. The method of claim 128, wherein the micro-dystrophin transgene comprises SEQ ID NO:5.

141. The method of any one of claims 48-140, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1.0×10^{9} to about 1×10^{16} vector genomes.

142. The method of any one of claims 48-140, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1.0×10^{9} to about 1×10^{15} vector genomes.

143. The method of any one of claims 48-140, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1.0×10^{9} to about 1×10^{14} vector genomes.

144. The method of any one of claims 48-140, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1.0×10^{9} to about 1×10^{13} vector genomes.

145. The method of any one of claims 48-140, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1.0×10^{9} to about 1×10^{12} vector genomes.

146. The method of any one of claims 48-140, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1.0×10^{9} to about 1×10^{11} vector genomes.

147. The method of any one of claims 48-140, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1.0×10^{9} to about 1×10^{10} vector genomes.

148. The method of claim 141, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1×10^{10} to 1×10^{16} vector genomes.

149. The method of claim 141, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1×10^{10} to 1×10^{15} vector genomes.

150. The method of claim 141, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1×10^{10} to 1×10^{14} vector genomes.

151. The method of claim 141, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1×10^{10} to 1×10^{13} vector genomes.

152. The method of claim 141, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1×10^{10} to 1×10^{12} vector genomes.

153. The method of claim 141, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1×10^{10} to 1×10^{11} vector genomes.

154. The method of claim 141, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1×10^{11} to 1×10^{16} vector genomes.

155. The method of claim 141, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1×10^{12} to 1×10^{16} vector genomes.

156. The method of claim 141, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1×10^{13} to 1×10^{16} vector genomes.

157. The method of claim 141, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1×10^{14} to 1×10^{16} vector genomes.

158. The method of claim 141, wherein the effective amount of the AAV particle in the intrathecal composition is from about 1×10^{15} to 1×10^{16} vector genomes.

159. The method of claim 141, wherein the effective amount of the AAV particle in the intrathecal composition is from about 2.5×10^{13} to 1×10^{14} vector genomes.

160. The method of any one of claims 48-159, wherein the intrathecal vector genome dose sufficient to provide a therapeutic response is about 2-fold, about 5-fold, about 10-fold, about 15-fold, about 20-fold, about 25-fold, about 30-fold, about 35-fold, about 40-fold, about 45-

fold, about 50-fold, about 55-fold, about 60-fold, about 65-fold, about 70-fold, about 75-fold, about 80-fold, about 85-fold, about 90-fold, about 95-fold, about 100-fold, about 150-fold, about 200-fold, about 250-fold lower than the vector genome dose of the intravenous composition sufficient to provide the same or substantially the same therapeutic response.

161. The method of claim 160, wherein the intrathecal vector genome dose sufficient to provide a therapeutic response is about 10-fold to 40-fold lower than the vector genome dose of the intravenous composition sufficient to provide the same or substantially the same therapeutic response.

162. The method of claim 160, wherein the intrathecal vector genome dose sufficient to provide a therapeutic response is about 20-fold lower than the vector genome dose of the intravenous composition sufficient to provide the same or substantially the same therapeutic response.

163. The method of claim 160, wherein the intrathecal vector genome dose sufficient to provide a therapeutic response is about 40-fold lower than the vector genome dose of the intravenous composition sufficient to provide the same or substantially the same therapeutic response.

164. The method of claim 160, wherein the intrathecal vector genome dose sufficient to provide a therapeutic response is about 100-fold lower than the vector genome dose of the intravenous composition sufficient to provide the same or substantially the same therapeutic response.

165. The method of any one of claims 48-159, wherein the intrathecal vector genome dose sufficient to provide a therapeutic response is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, at least about 50-fold, at least about 55-fold, at least about 60-fold, at least about 65-fold, at least about 70-fold, at least about 75-fold, at least about 80-fold, at least about 85-fold, at least about 90-fold, at least about 95-fold, at least about 100-fold, at least about 150-fold, at least about 200-fold, at least about 250-fold, lower than the vector genome dose of the intravenous composition sufficient to provide the same or substantially the same therapeutic response.

166. The method of claim 165, wherein the intrathecal vector genome dose sufficient to provide a therapeutic response is about 10-fold lower than the vector genome dose of the intravenous composition sufficient to provide the same or substantially the same therapeutic response.

167. The method of claim 165, wherein the intrathecal vector genome dose sufficient to provide a therapeutic response is about 20-fold lower than the vector genome dose of the intravenous composition sufficient to provide the same or substantially the same therapeutic response.

168. The method of claim 165, wherein the intrathecal vector genome dose sufficient to provide a therapeutic response is about 40-fold lower than the vector genome dose of the intravenous composition sufficient to provide the same or substantially the same therapeutic response.

169. The method of claim 165, wherein the intrathecal vector genome dose sufficient to provide a therapeutic response is about 100-fold lower than the vector genome dose of the intravenous composition sufficient to provide the same or substantially the same therapeutic response.

FIG. 1A

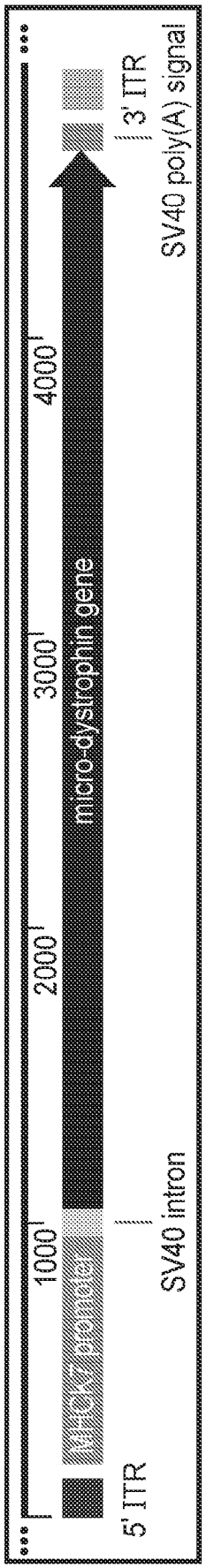
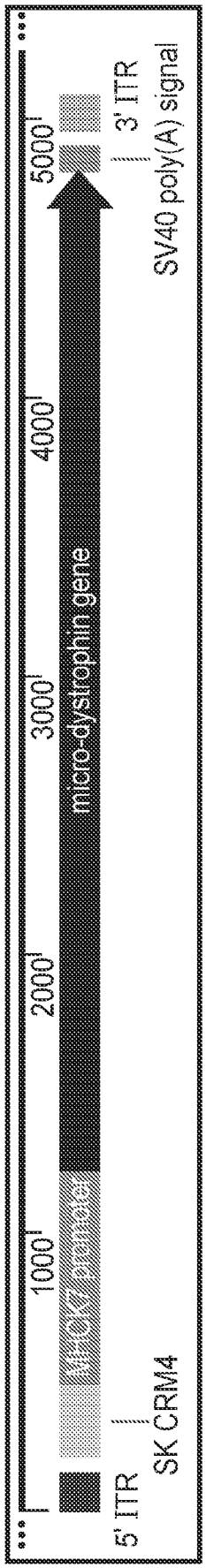

INS1201
1000
2000
3000
4000
5' ITR
MCK promoter
SV40 intron
micro-dystrophin gene
3' ITR
SV40 poly(A) signal

FIG. 1B


INS1212
1000
2000
3000
4000
5000
5' ITR
SK CRM4
MCK promoter
micro-dystrophin gene
3' ITR
SV40 poly(A) signal

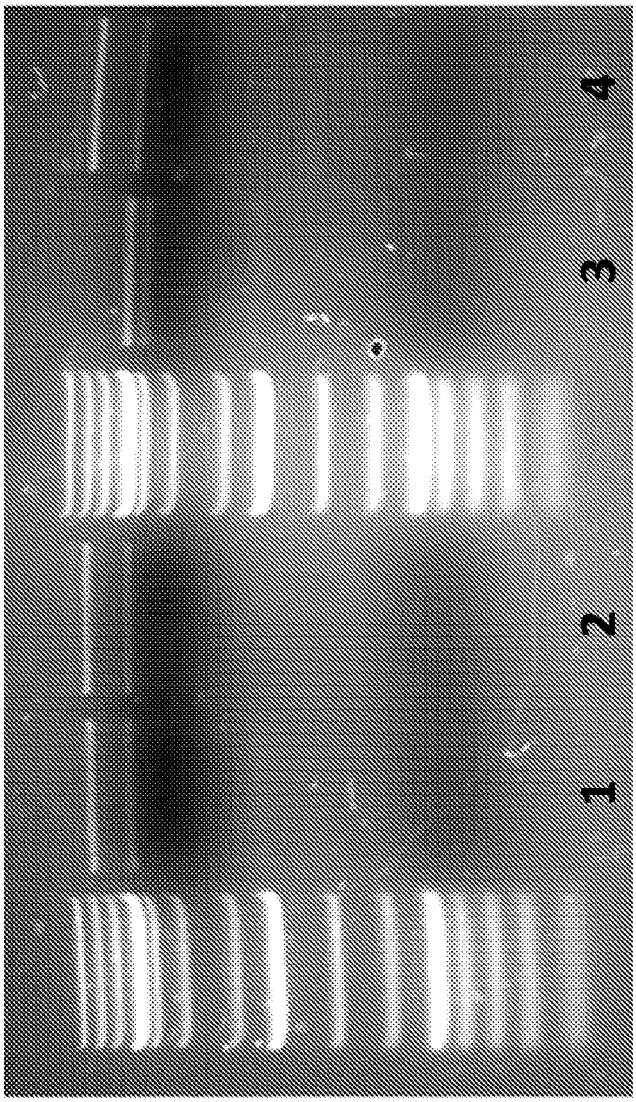


FIG. 1C

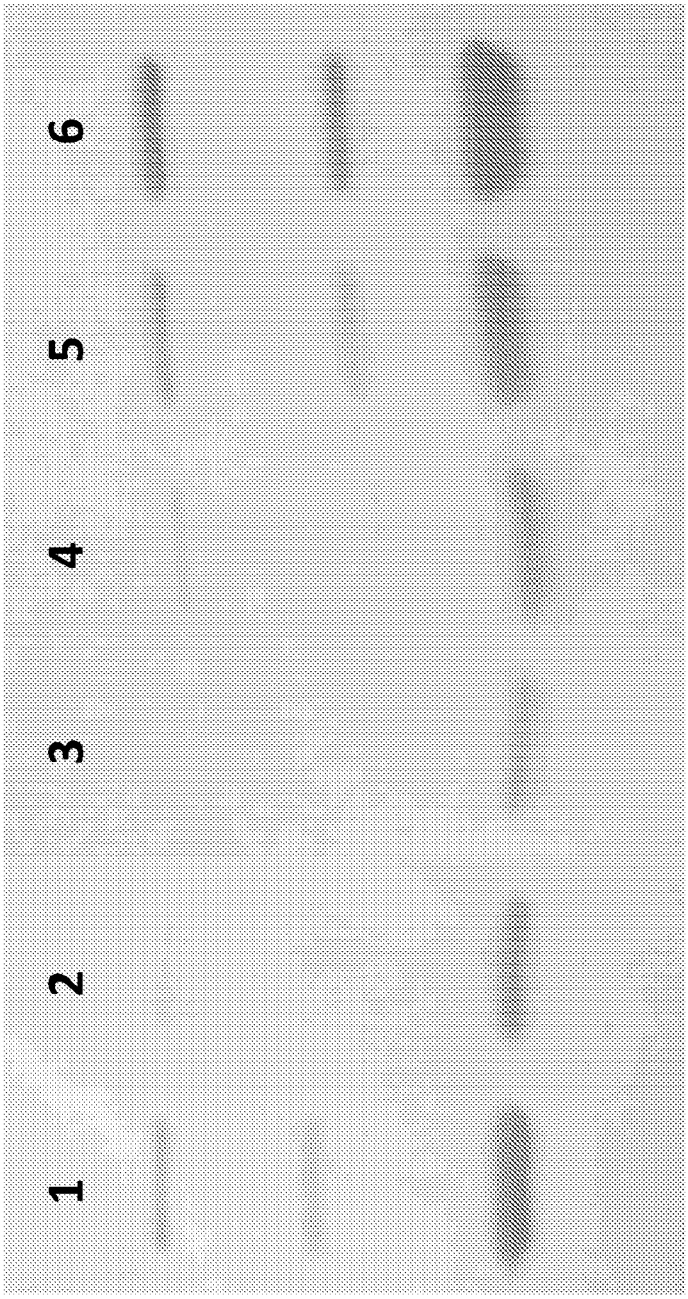


FIG. 2

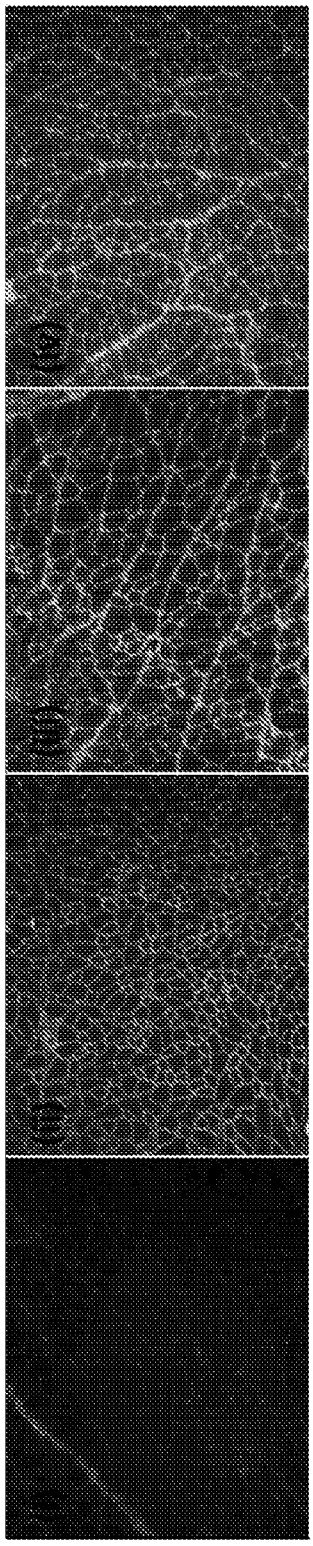


FIG. 3A

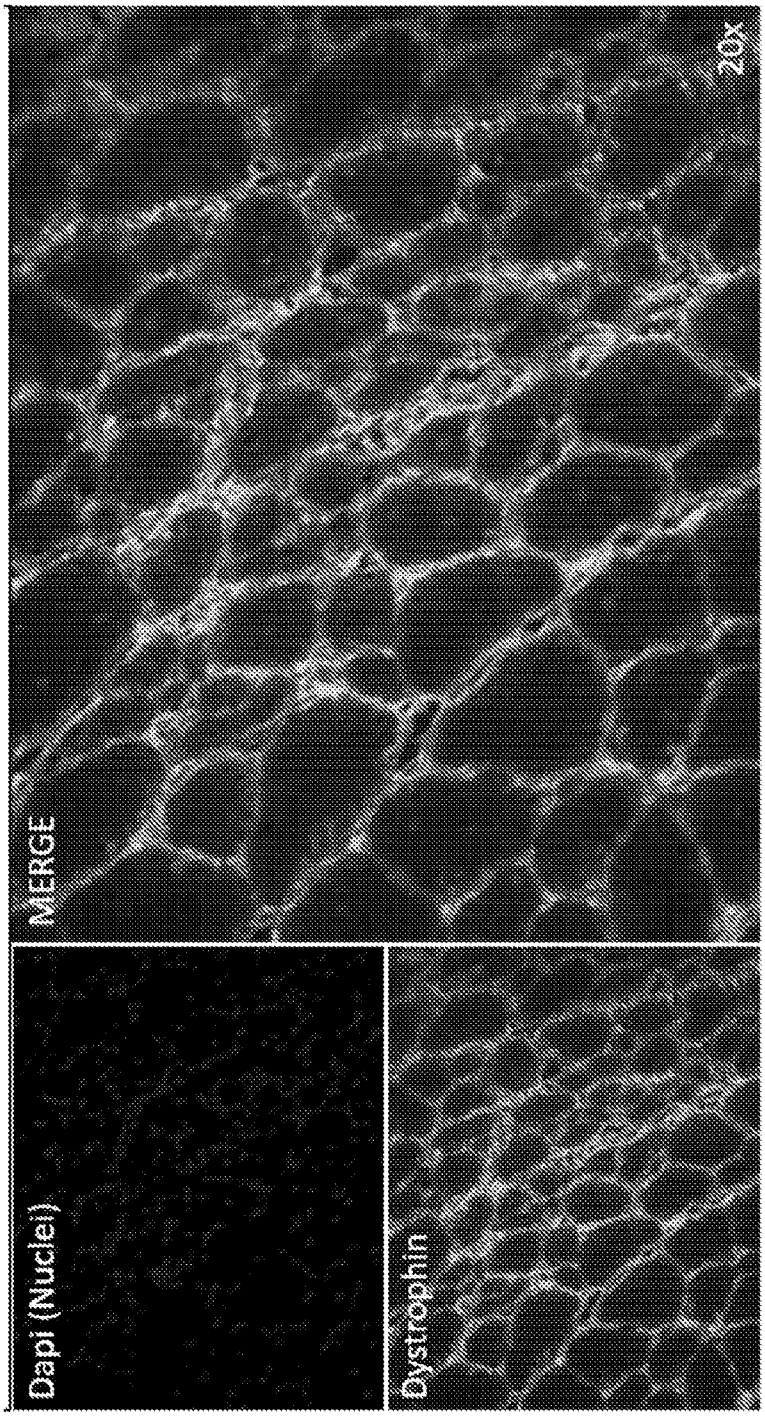
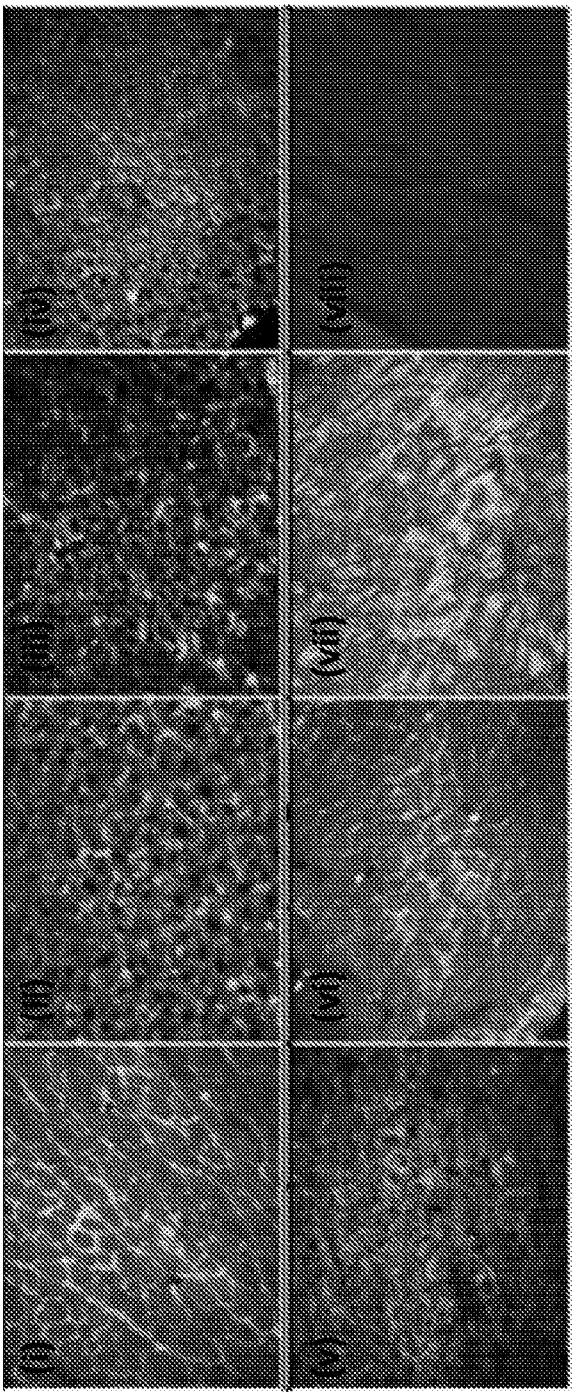


FIG. 3B

FIG. 4A



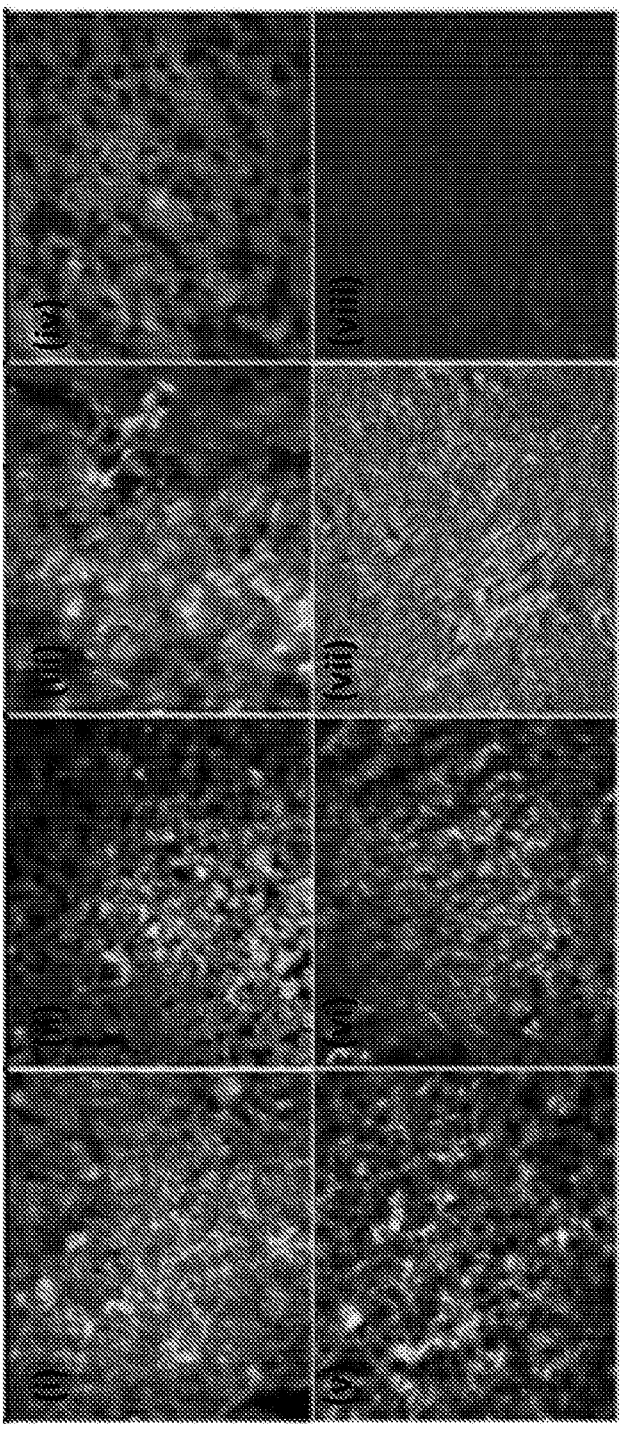
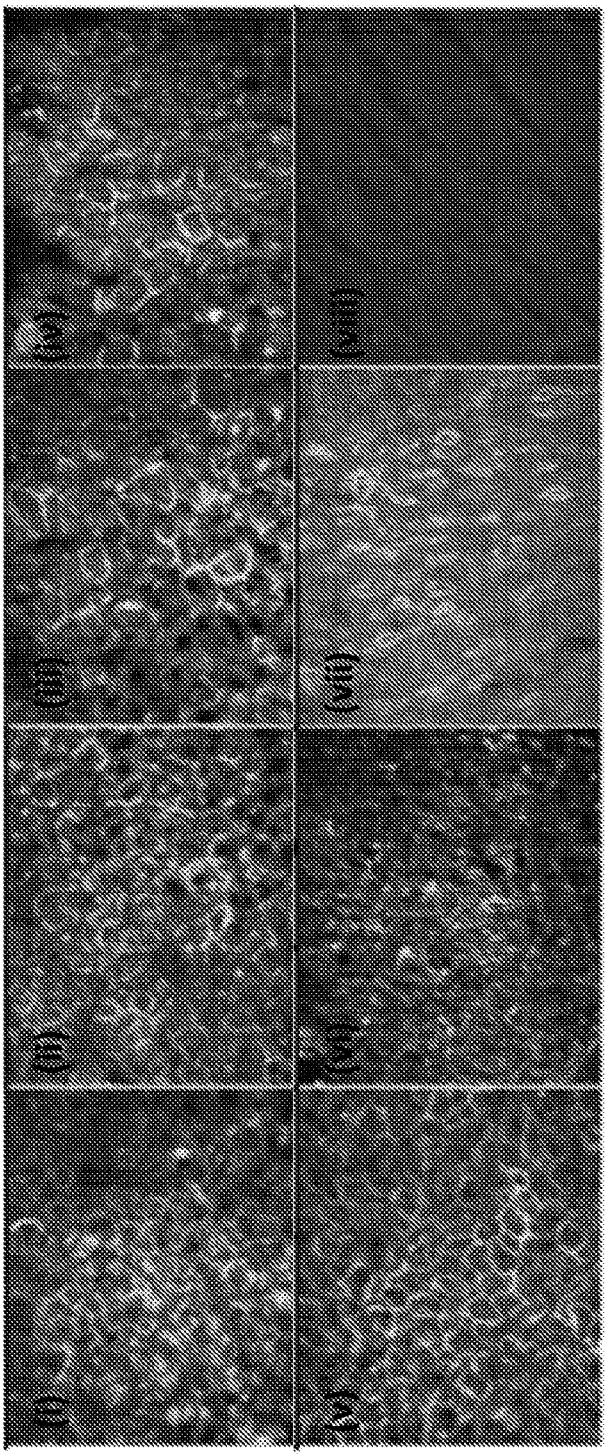


FIG. 4B

FIG. 5



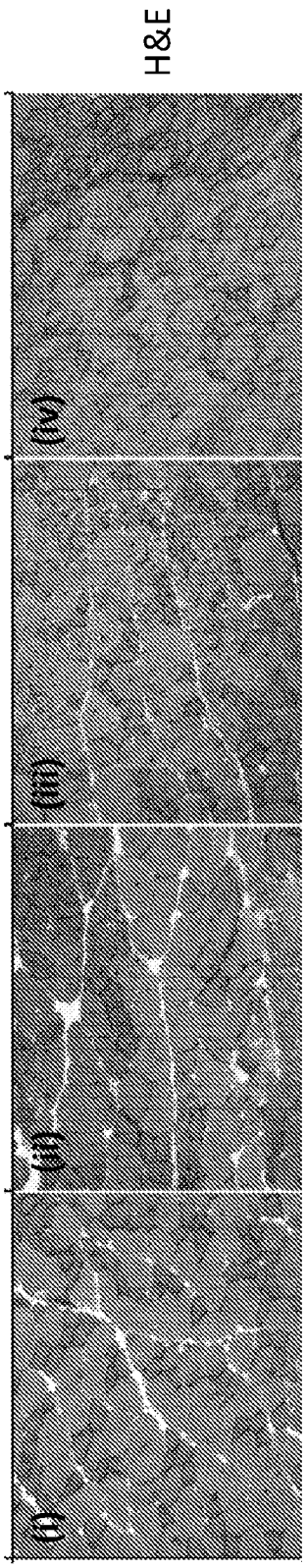


FIG. 6A

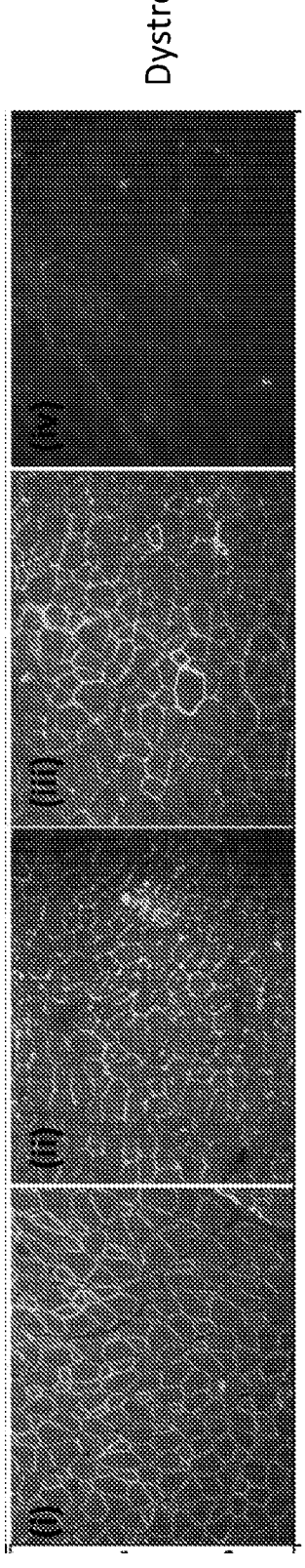


FIG. 6B

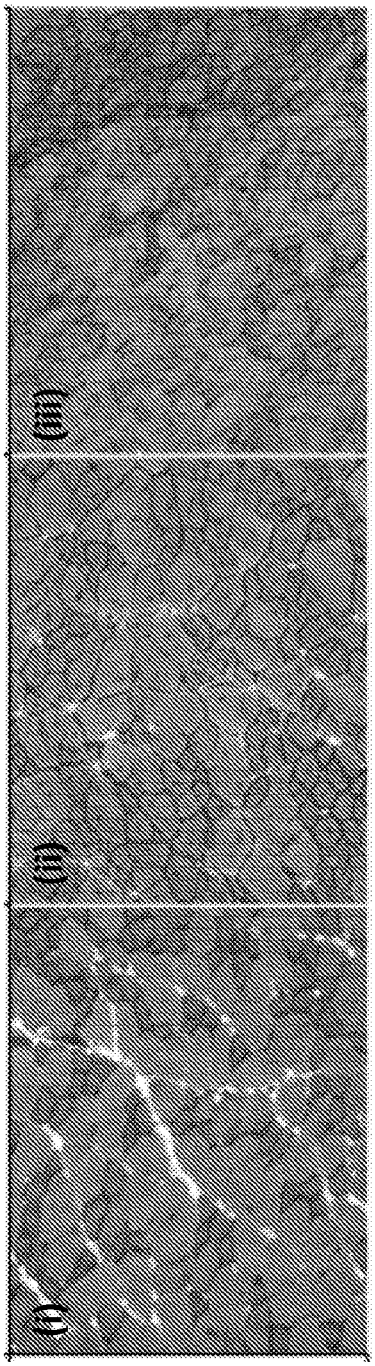


FIG. 7A

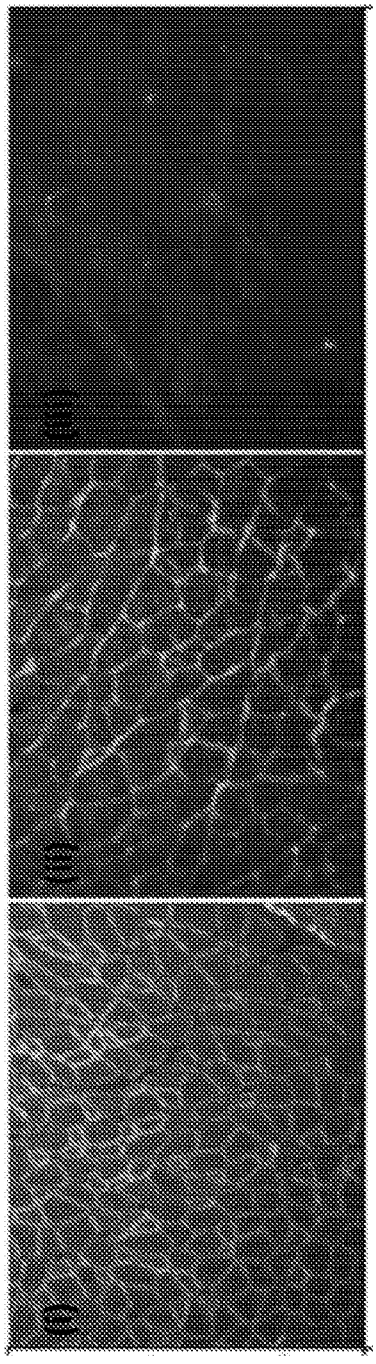


FIG. 7B

FIG. 8A

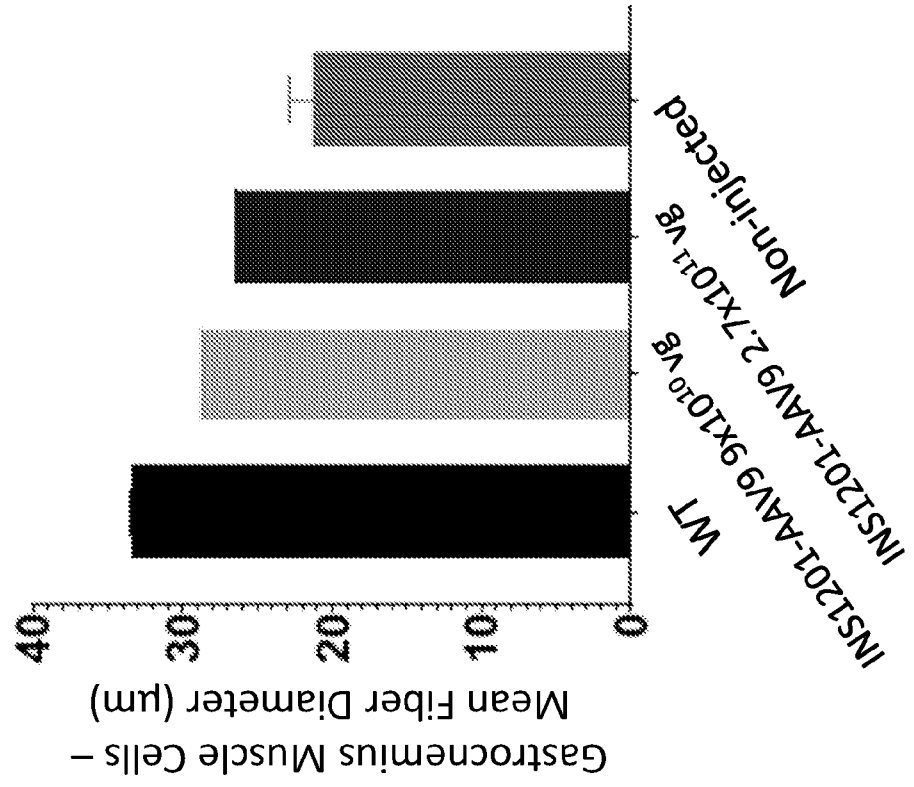

Gastrocnemius Muscle Cells –
Mean Fiber Diameter (μm)
0
10
20
30
40
WT
INS1201-AAV9 9X10¹⁰ VG
INS1201-AAV9 2.7X10¹¹ VG
Non-injected

FIG. 8B

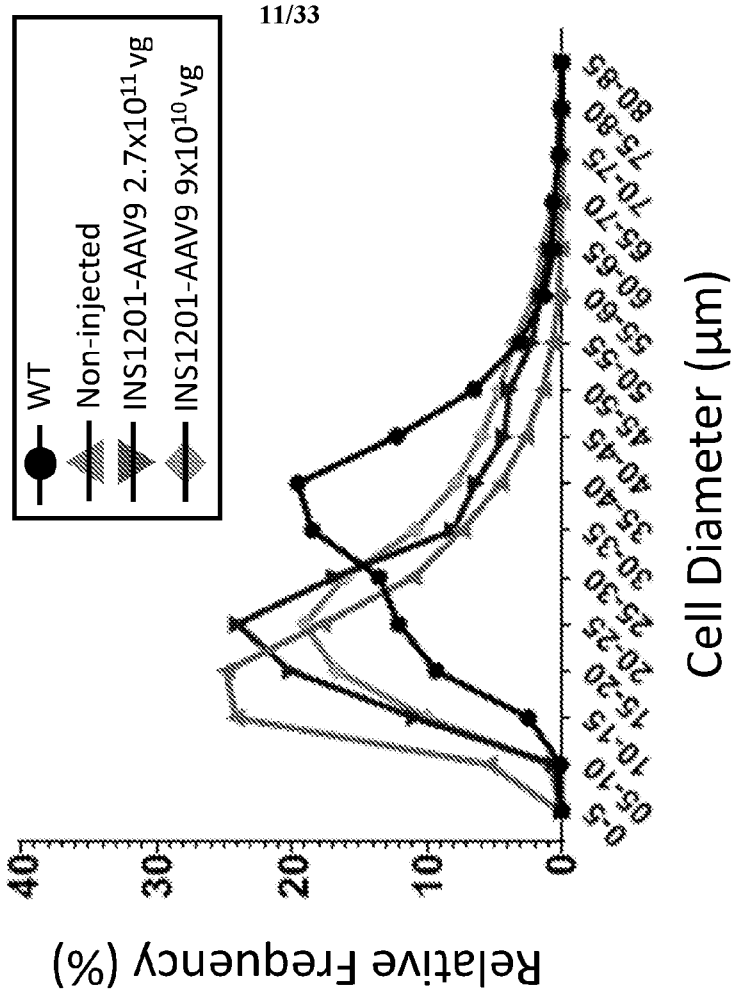

Relative Frequency (%)
0
10
20
30
40
0-5
05-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
50-55
55-60
60-65
65-70
70-75
75-80
80-85
Cell Diameter (μm)
WT
Non-injected
INS1201-AAV9 2.7x10¹¹ vg
INS1201-AAV9 9x10¹⁰ vg

FIG. 8C

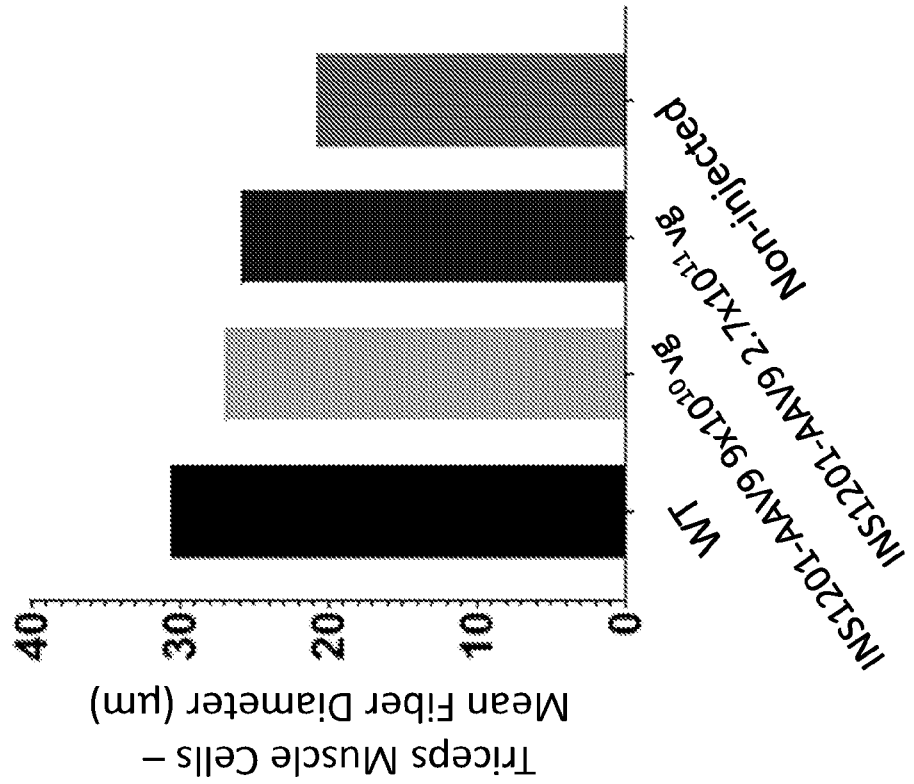

Triceps Muscle Cells –
Mean Fiber Diameter (μm)
0
10
20
30
40
WT
INS1201-AAV9 9X10¹⁰ VG
INS1201-AAV9 2.7X10¹¹ VG
Non-injected

FIG. 8D

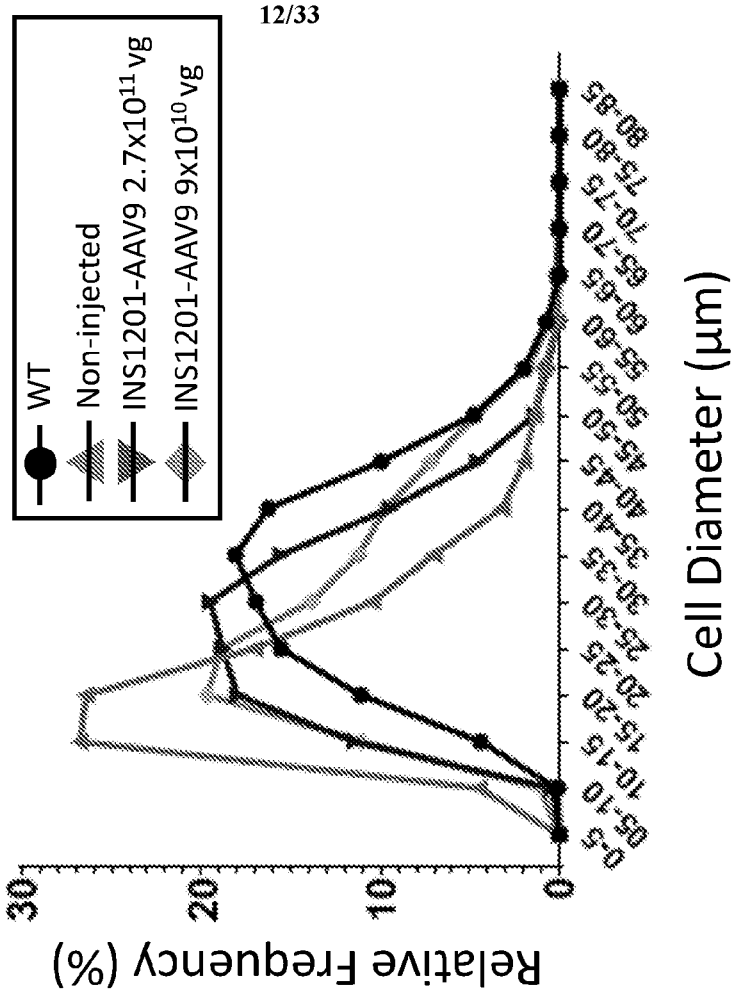

Relative Frequency (%)
0
10
20
30
Cell Diameter (μm)
0-5
05-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
50-55
55-60
60-65
65-70
70-75
75-80
80-85
WT
Non-injected
INS1201-AAV9 2.7x10¹¹vg
INS1201-AAV9 9x10¹⁰vg

FIG. 8E

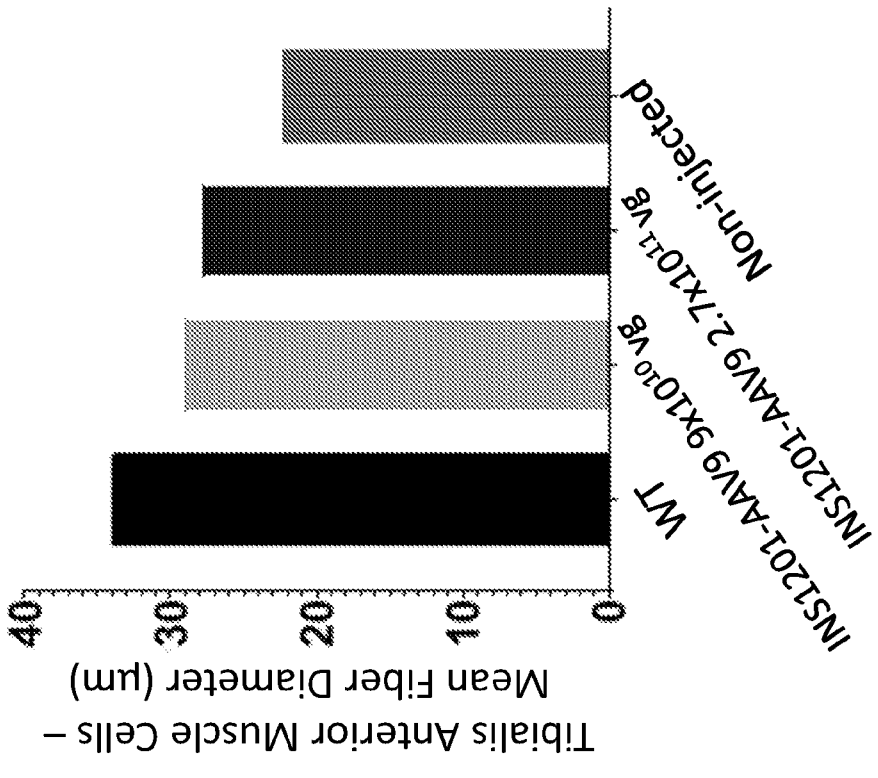

Tibialis Anterior Muscle Cells –
Mean Fiber Diameter (μm)
0
10
20
30
40
WT
INS1201-AAV9 9X10^10 vg
INS1201-AAV9 2.7X10^11 vg
Non-injected

FIG. 8F

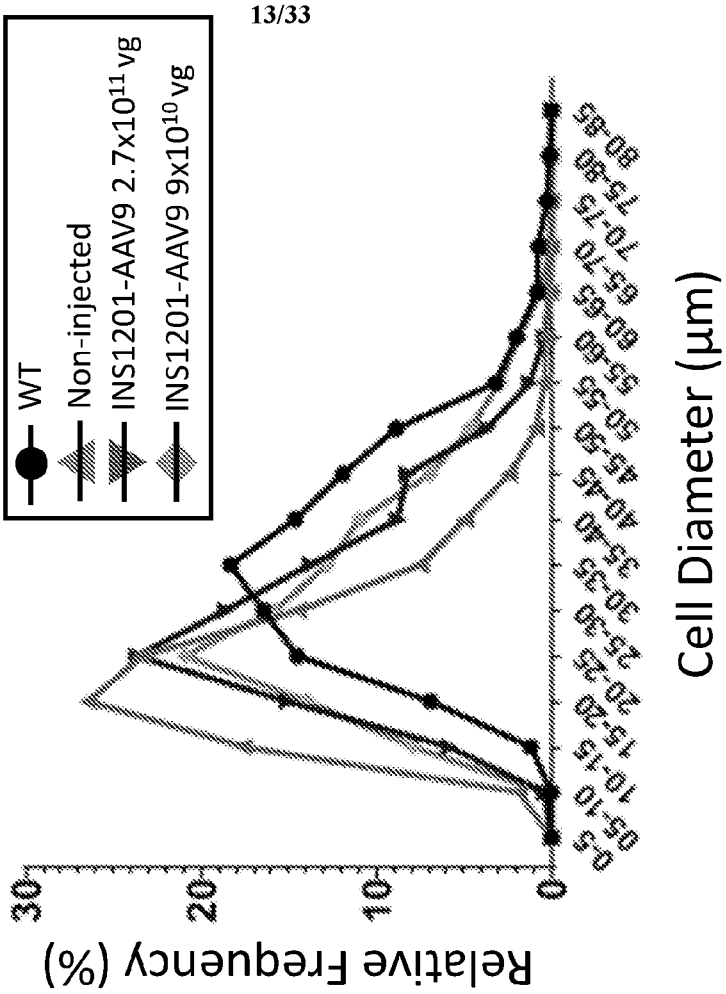

Relative Frequency (%)
0
10
20
30
Cell Diameter (μm)
0-5
05-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
50-55
55-60
60-65
65-70
70-75
75-80
80-85
WT
Non-injected
INS1201-AAV9 2.7x10^11 vg
INS1201-AAV9 9x10^10 vg

FIG. 8G

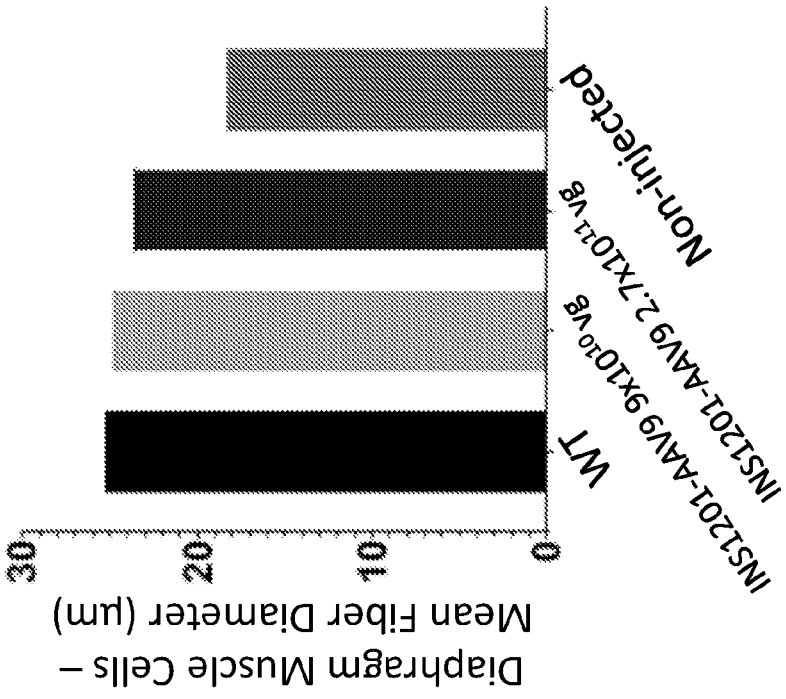

Diaphragm Muscle Cells –
Mean Fiber Diameter (μm)
0
10
20
30
WT
INS1201-AAV9 9X10¹⁰ vg
INS1201-AAV9 2.7X10¹¹ vg
Non-injected

FIG. 8H

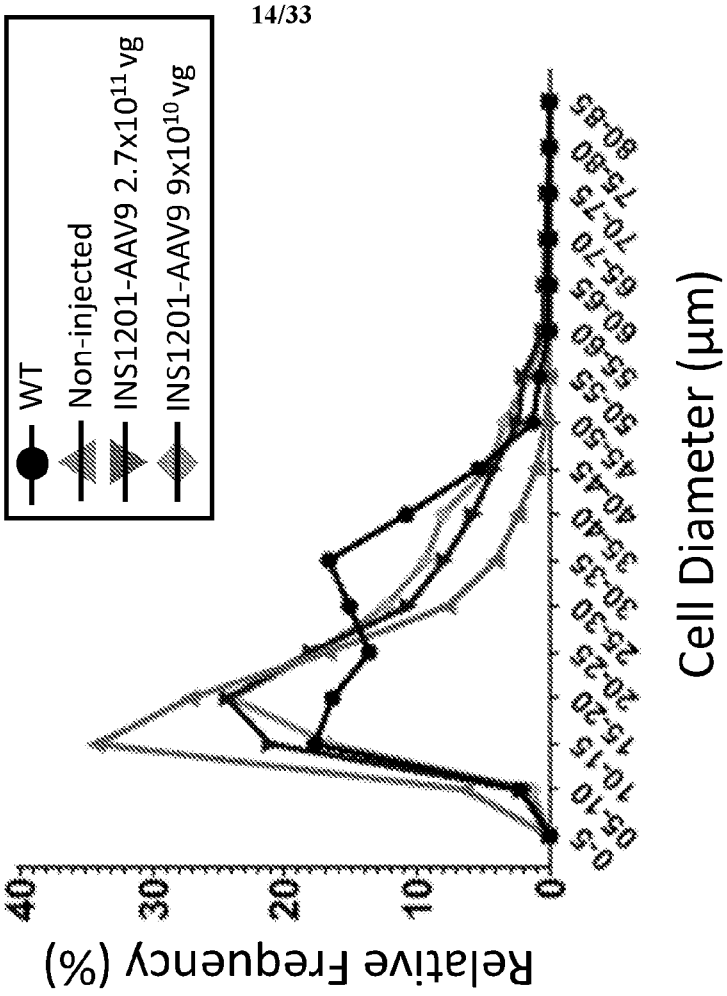

WT
Non-injected
INS1201-AAV9 2.7x10¹¹vg
INS1201-AAV9 9x10¹⁰vg
Relative Frequency (%)
0
10
20
30
40
0-5
05-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
50-55
55-60
60-65
65-70
70-75
75-80
80-85
Cell Diameter (μm)

FIG. 9A

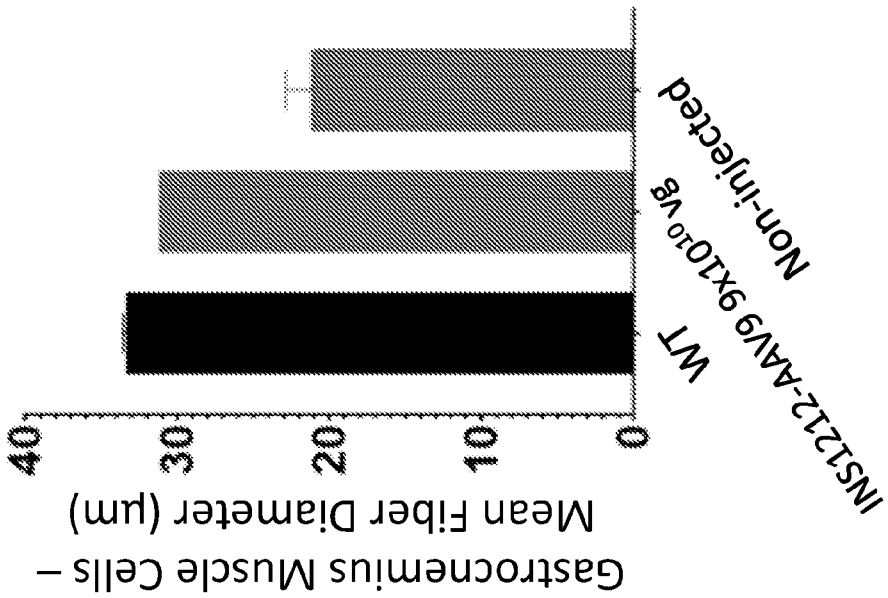

Gastrocnemius Muscle Cells –
Mean Fiber Diameter (μm)
0
10
20
30
40
WT
INS1212-AAV9 9x10¹⁰ vg
Non-injected

FIG. 9B

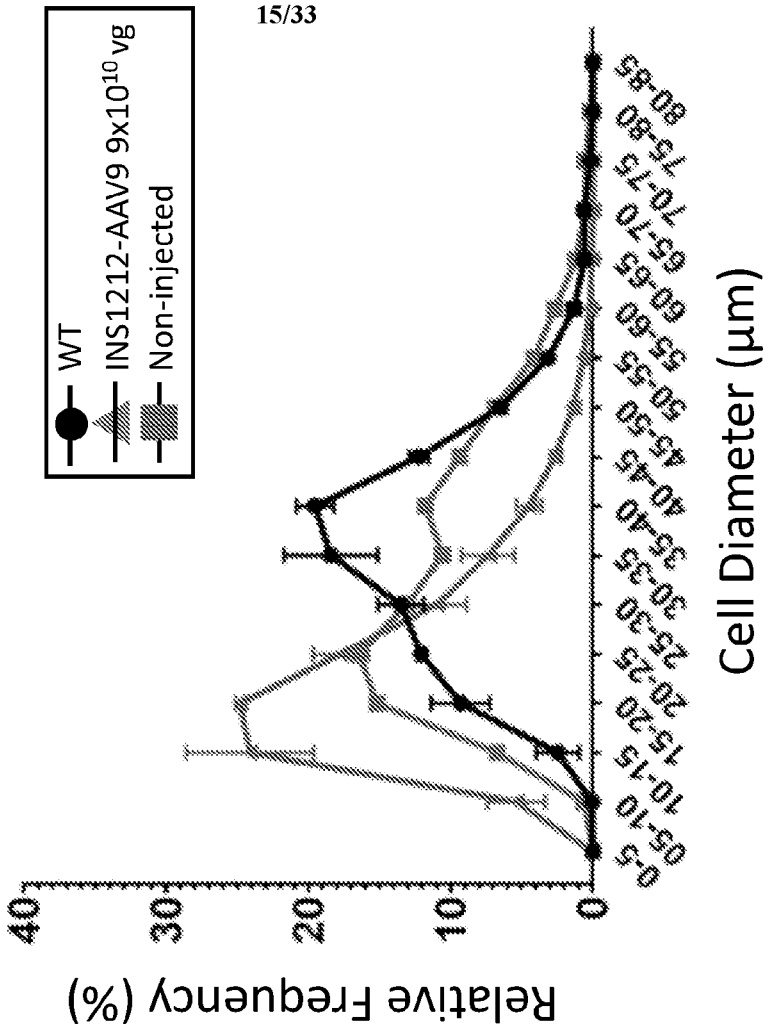

Relative Frequency (%)
0
10
20
30
40
Cell Diameter (μm)
0-5
05-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
50-55
55-60
60-65
65-70
70-75
75-80
80-85
WT
INS1212-AAV9 9x10¹⁰ vg
Non-injected

FIG. 10A

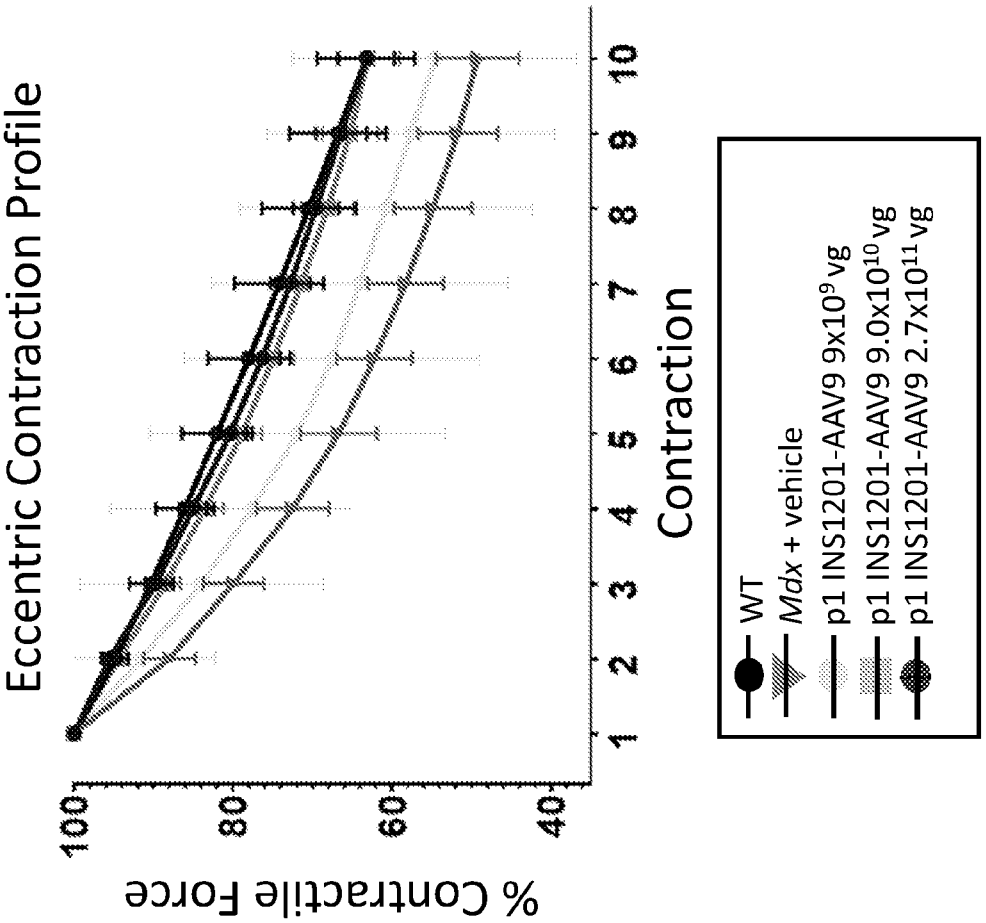


FIG. 10B

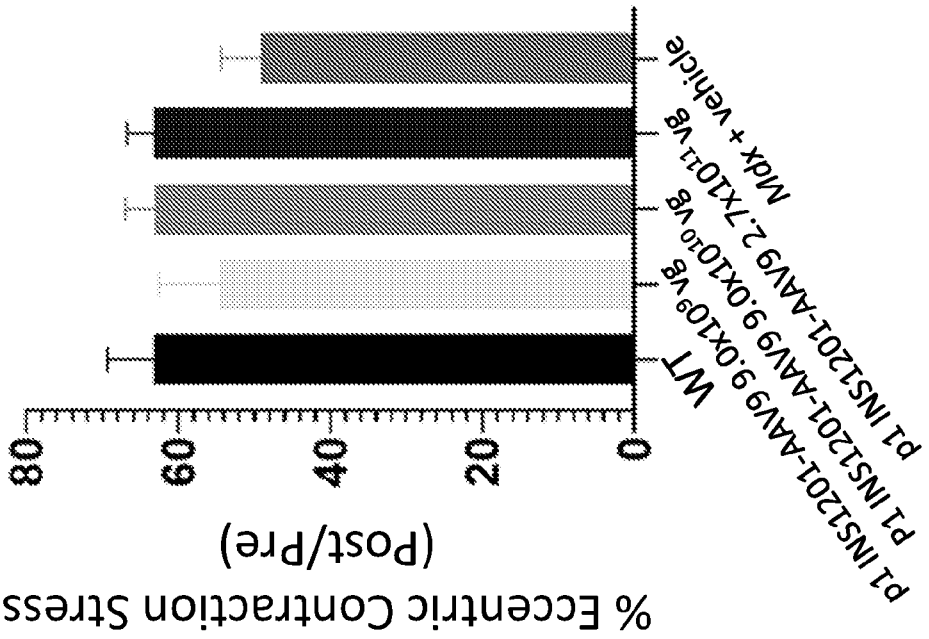


FIG. 10C

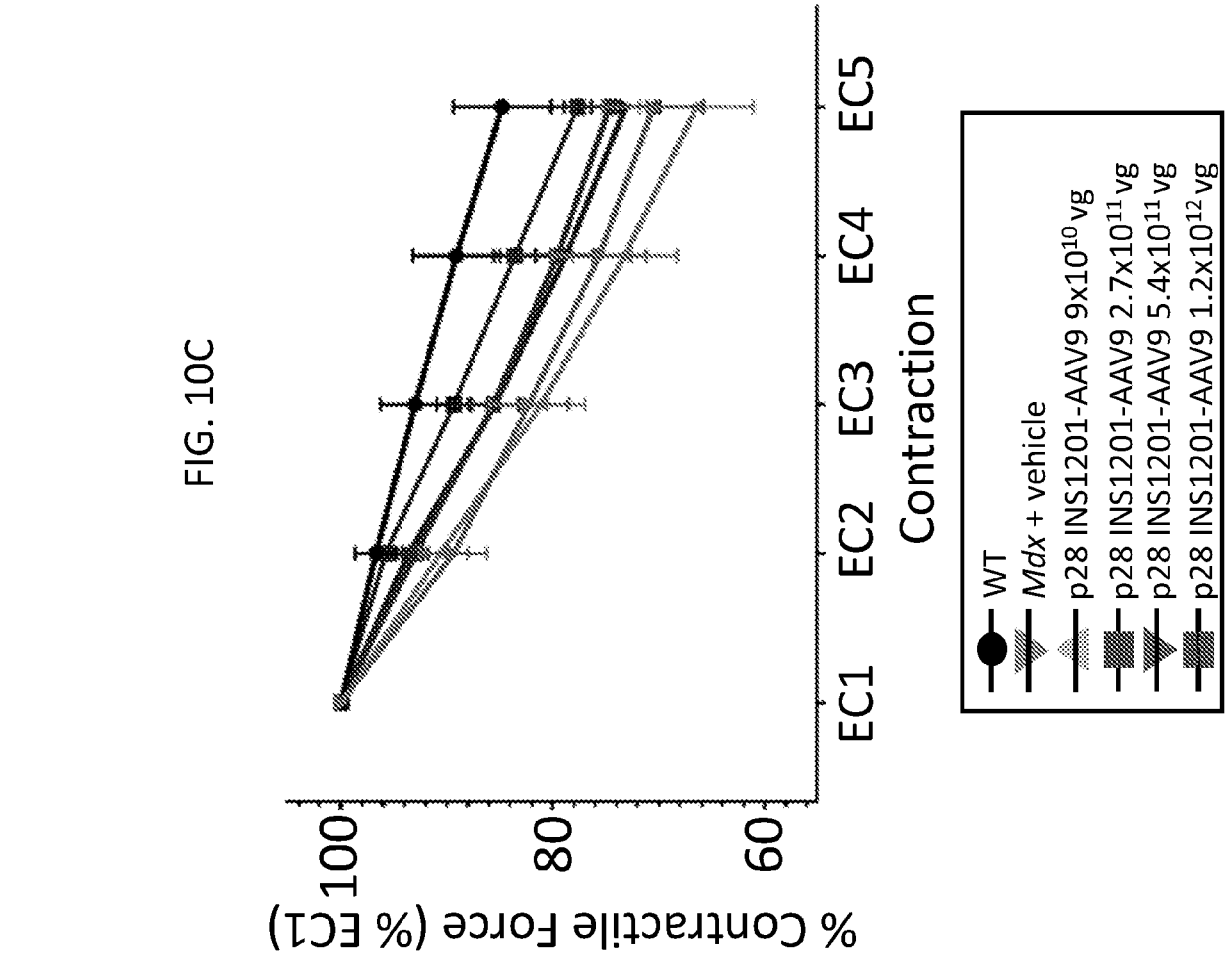


FIG. 10D

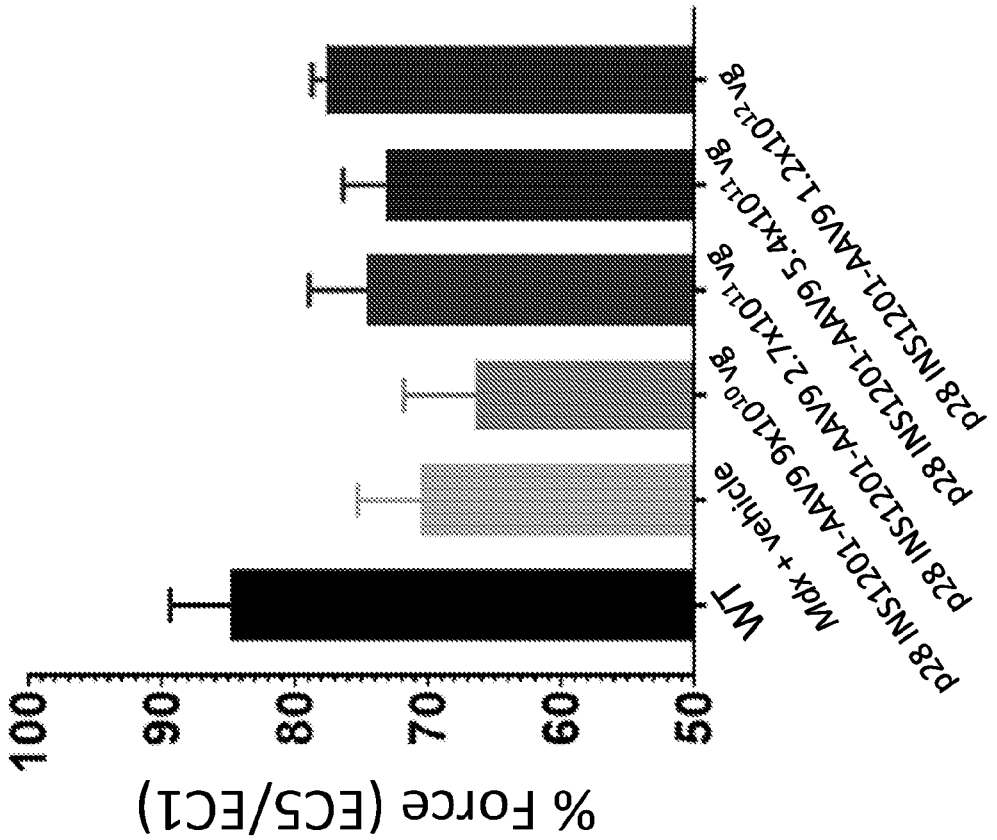


FIG. 10E

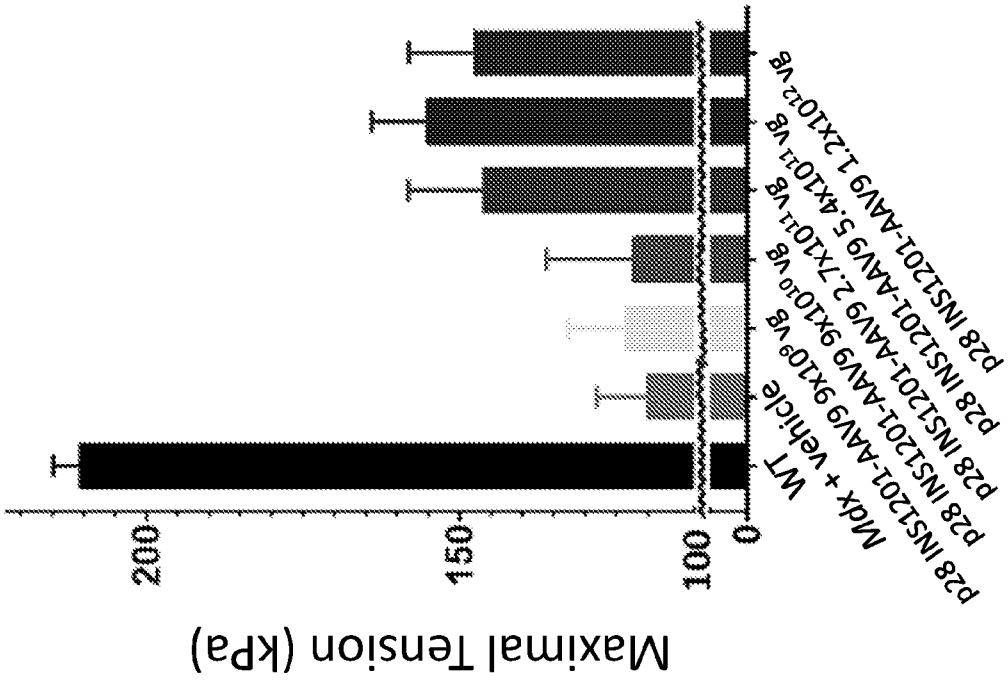
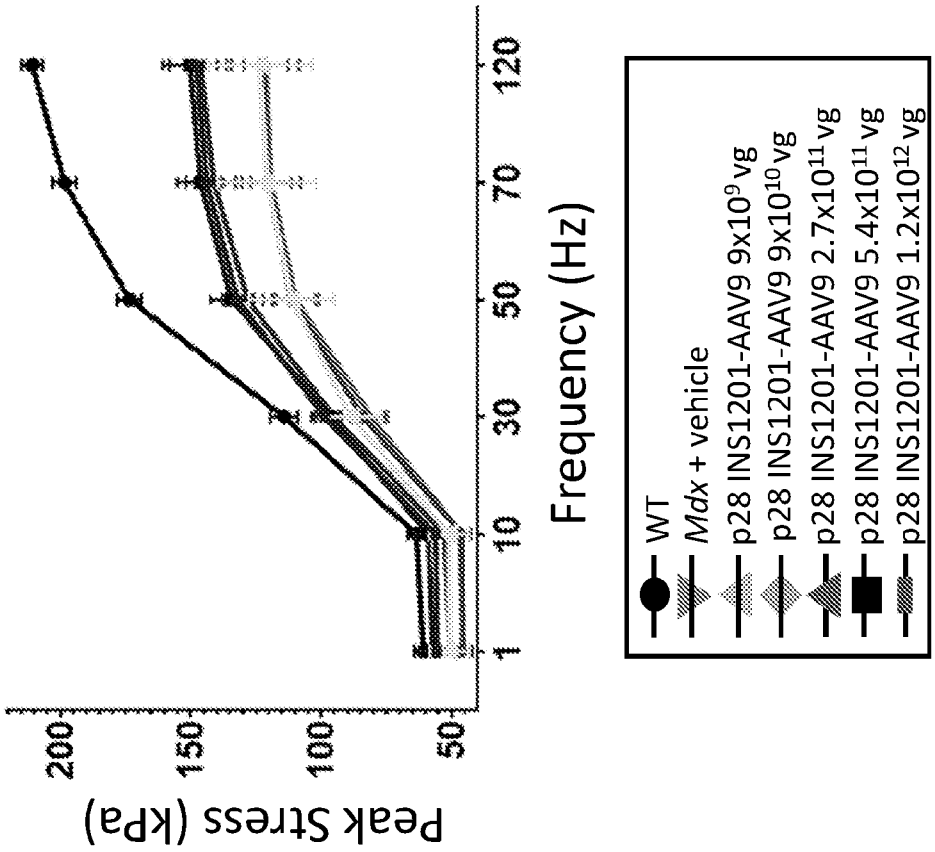


FIG. 10F



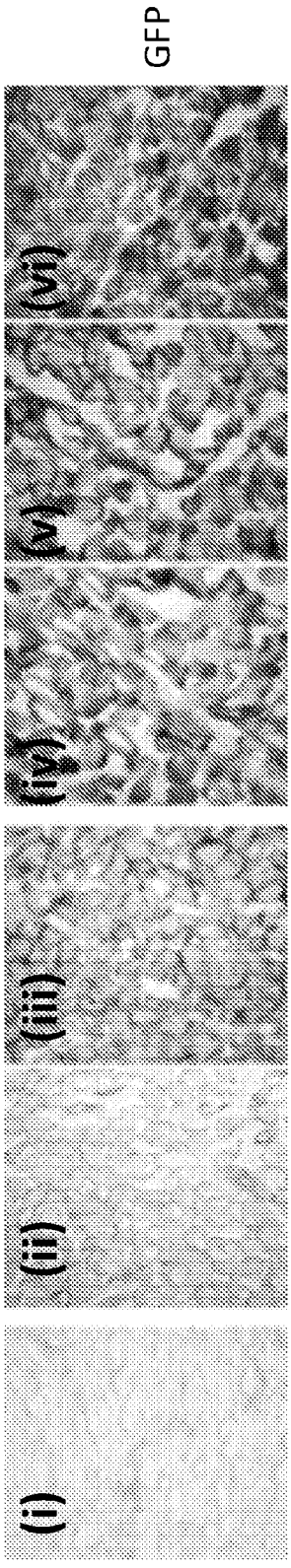


FIG. 11A

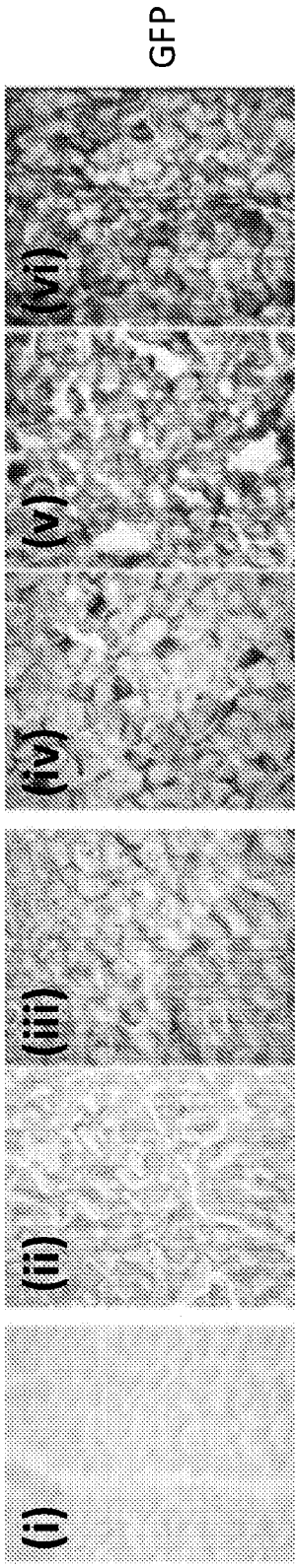


FIG. 11B

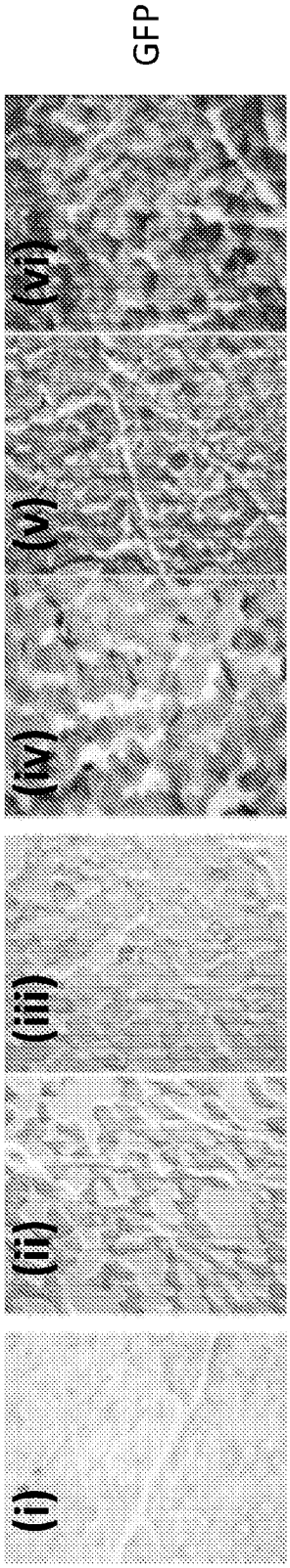


FIG. 11C

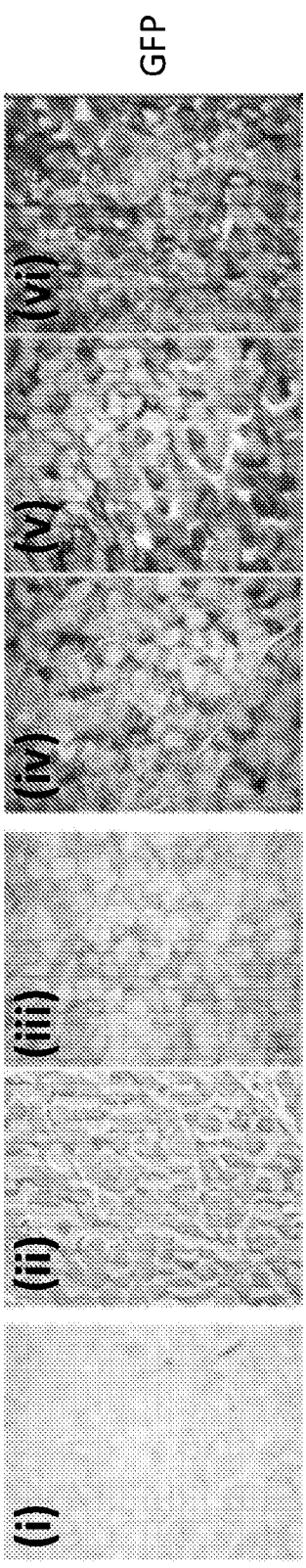


FIG. 11D

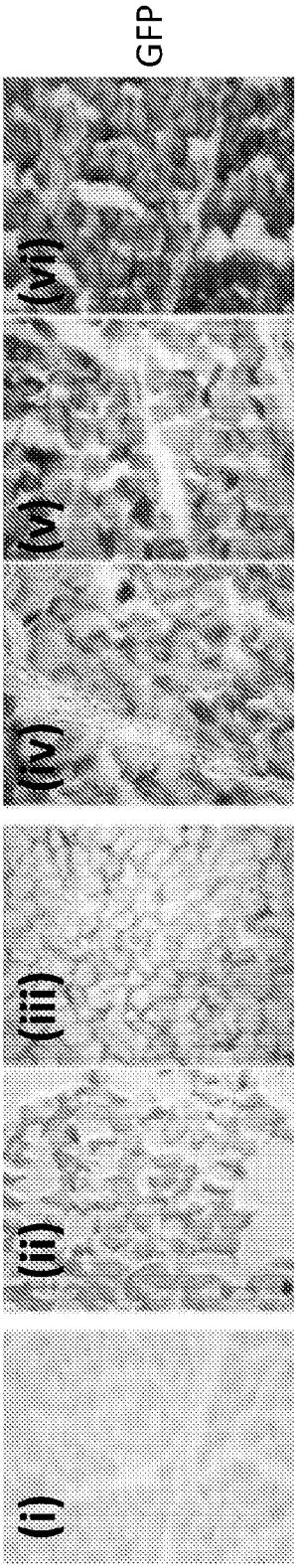


FIG. 11E

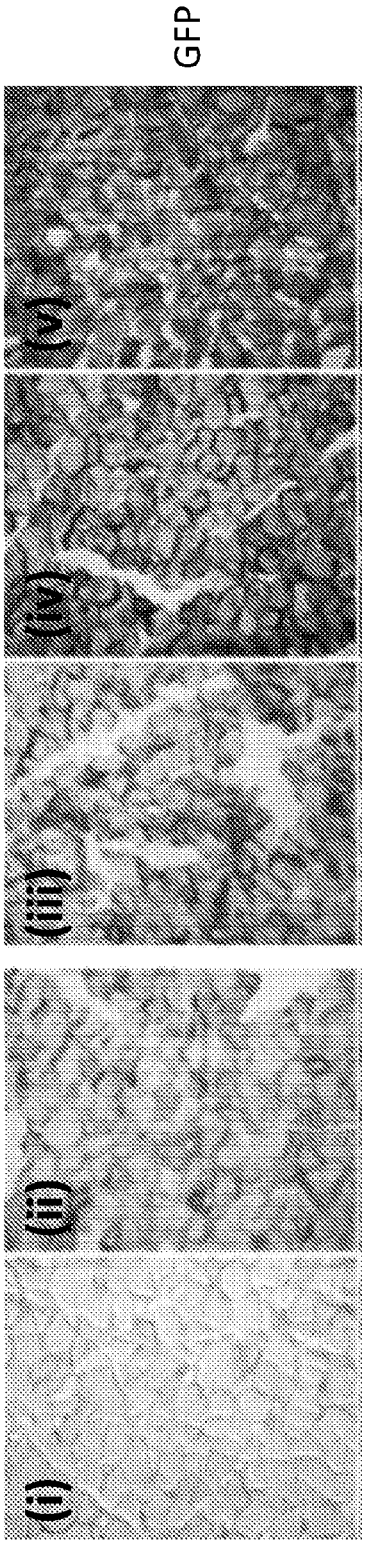


FIG. 11F

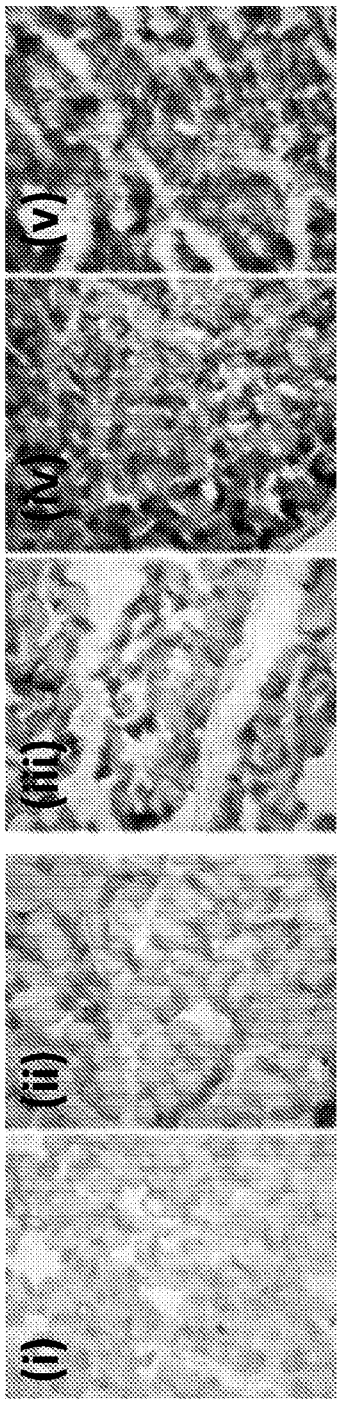


FIG. 11G

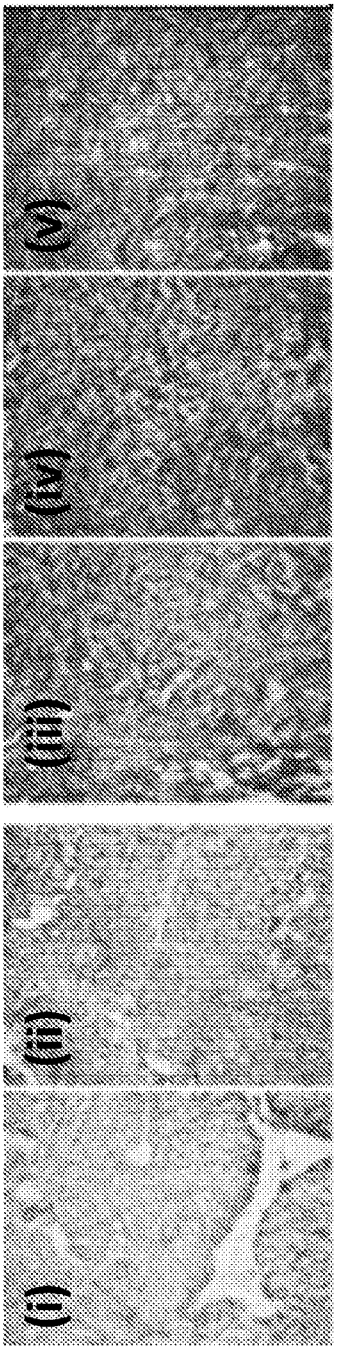


FIG. 11H

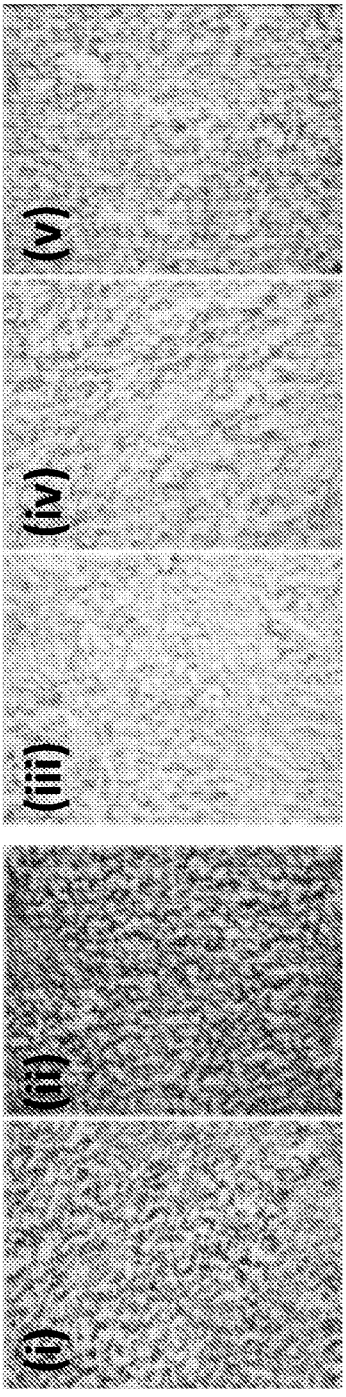


Figure 11I

FIG. 12A

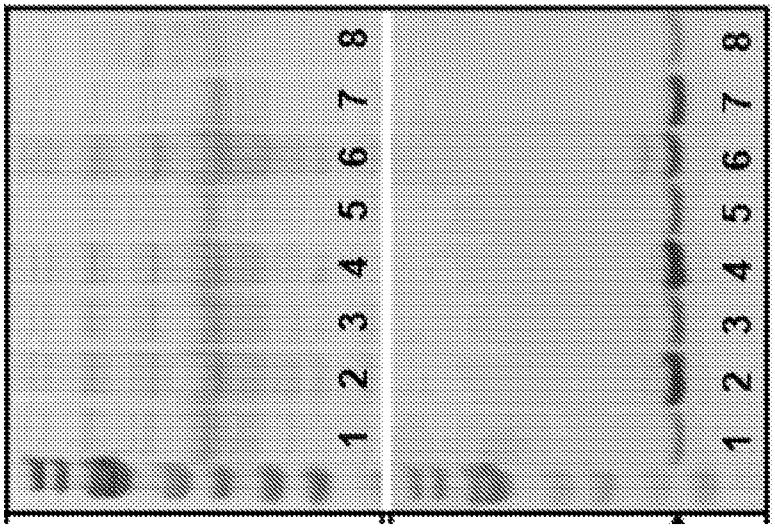
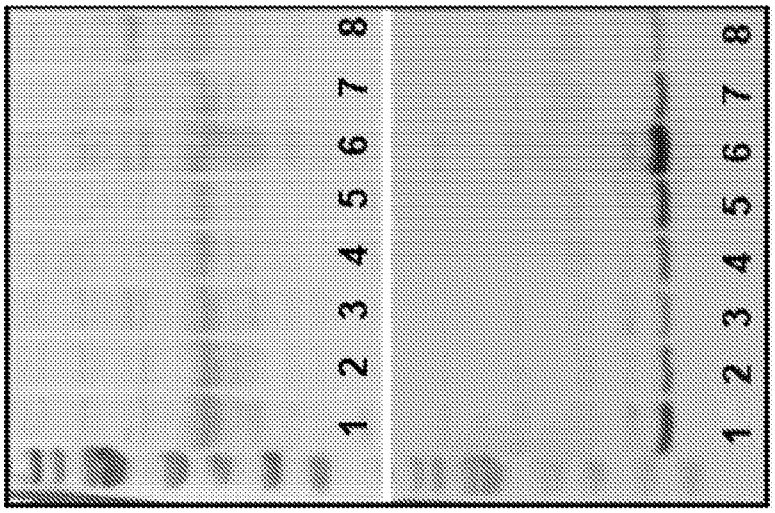


FIG. 12B



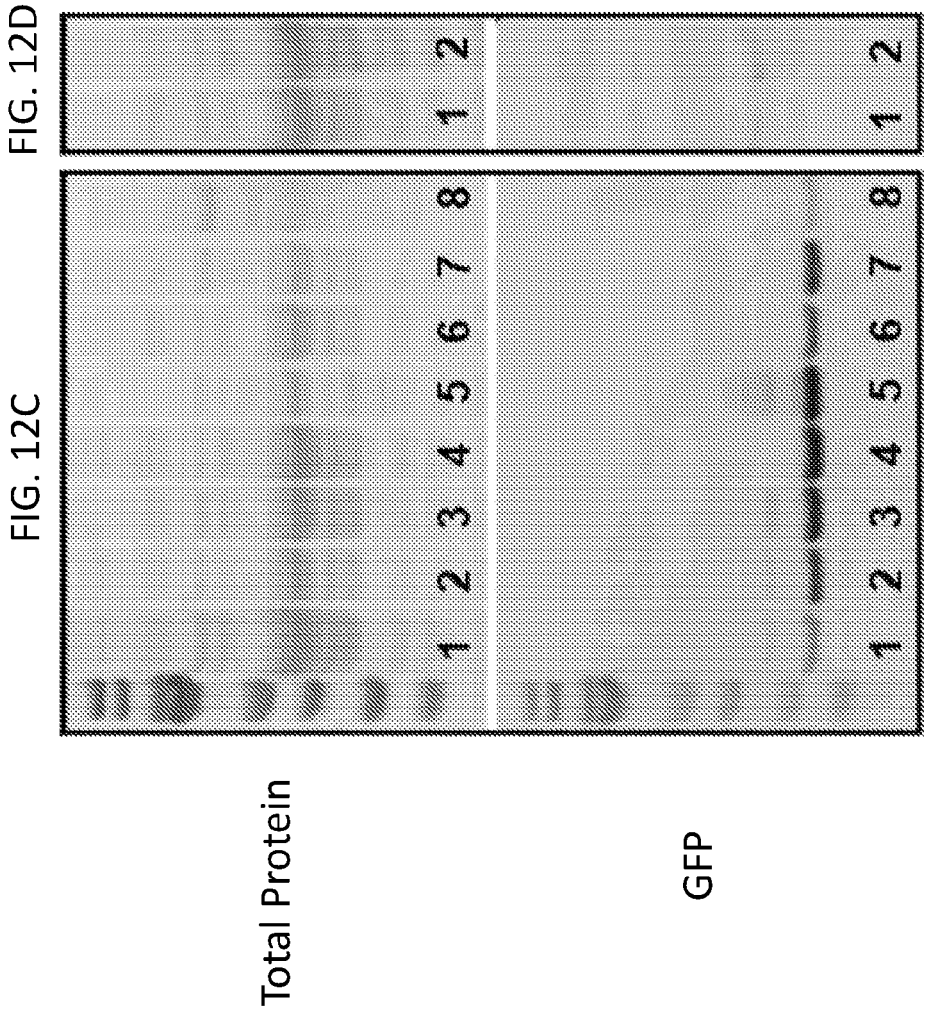


FIG. 12C
FIG. 12D
Total Protein
GFP
1 2 3 4 5 6 7 8
1 2
1 2 3 4 5 6 7 8
1 2

FIG. 13

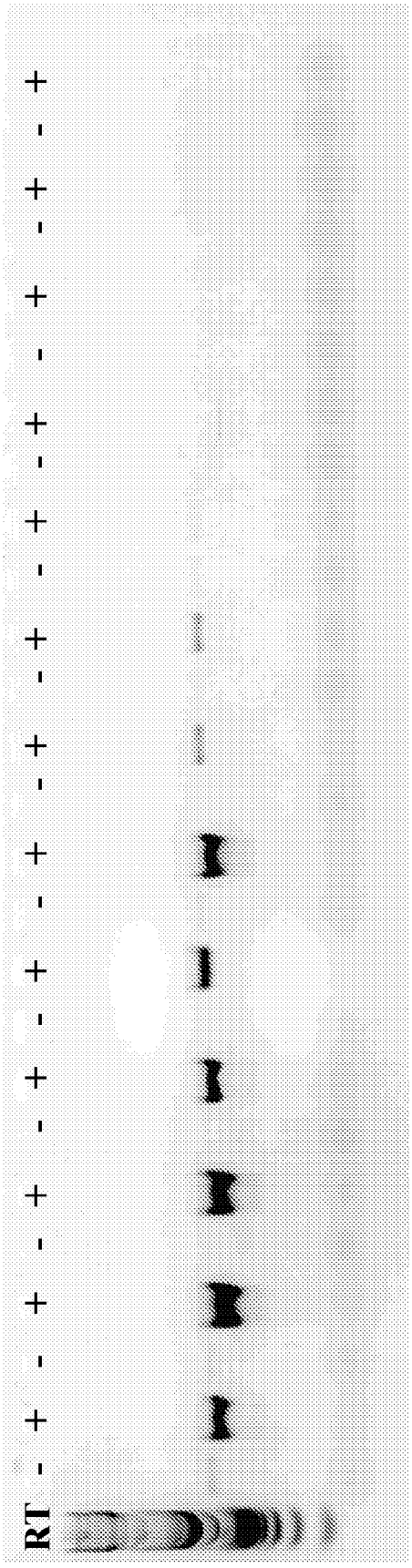
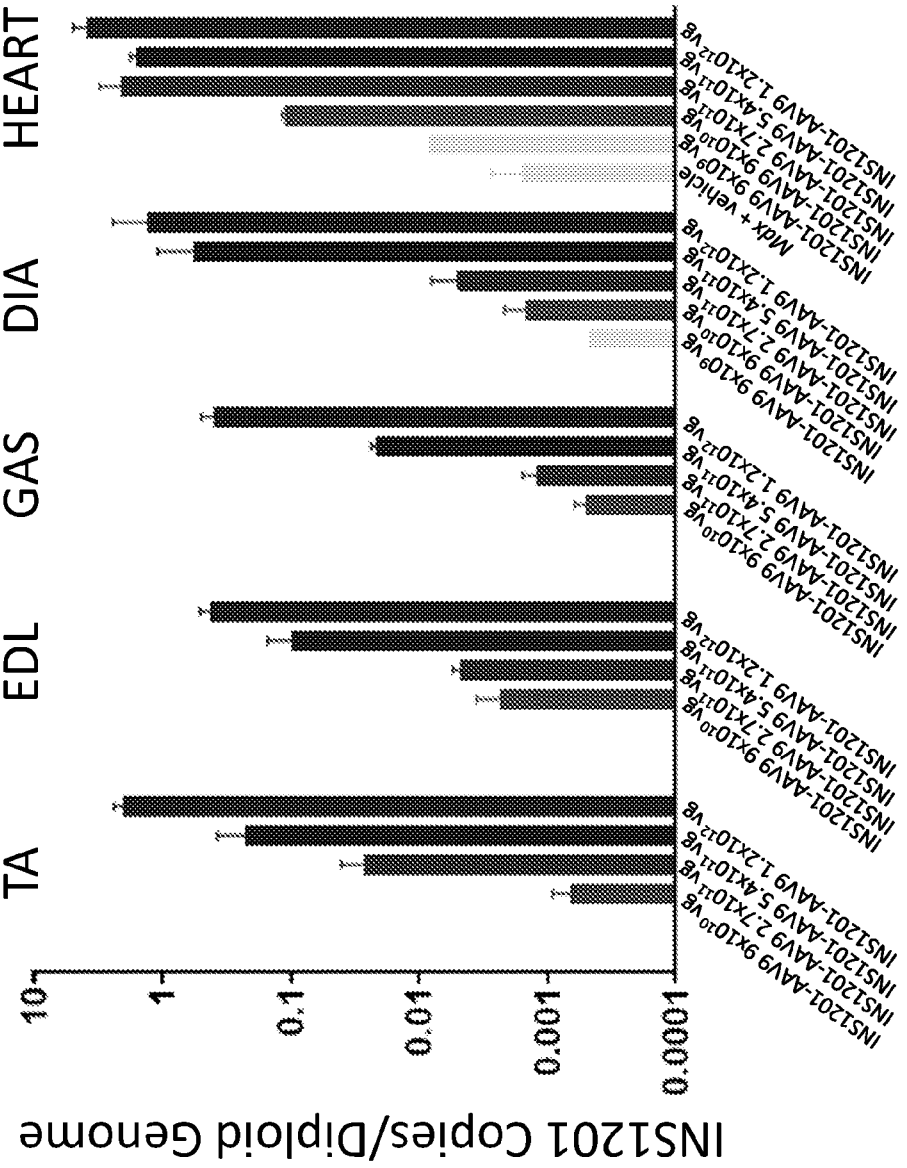


FIG. 14

NTD: N-terminal domain; H: Hinge region; numbered oval: spectrin repeat (SR); CRD: cysteine rich domain; CTD: C-terminal domain

FIG. 15


INS1201 Copies/Diploid Genome
10
1
0.1
0.01
0.001
0.0001
TA
EDL
GAS
DIA
HEART
INS1201-AAV9 9x10¹⁰ vg
INS1201-AAV9 2.7x10¹¹ vg
INS1201-AAV9 5.4x10¹¹ vg
INS1201-AAV9 1.2x10¹² vg
Mdx + vehicle
INS1201-AAV9 9x10⁹ vg
INS1201-AAV9 2.7x10¹⁰ vg
INS1201-AAV9 5.4x10¹¹ vg
INS1201-AAV9 1.2x10¹² vg

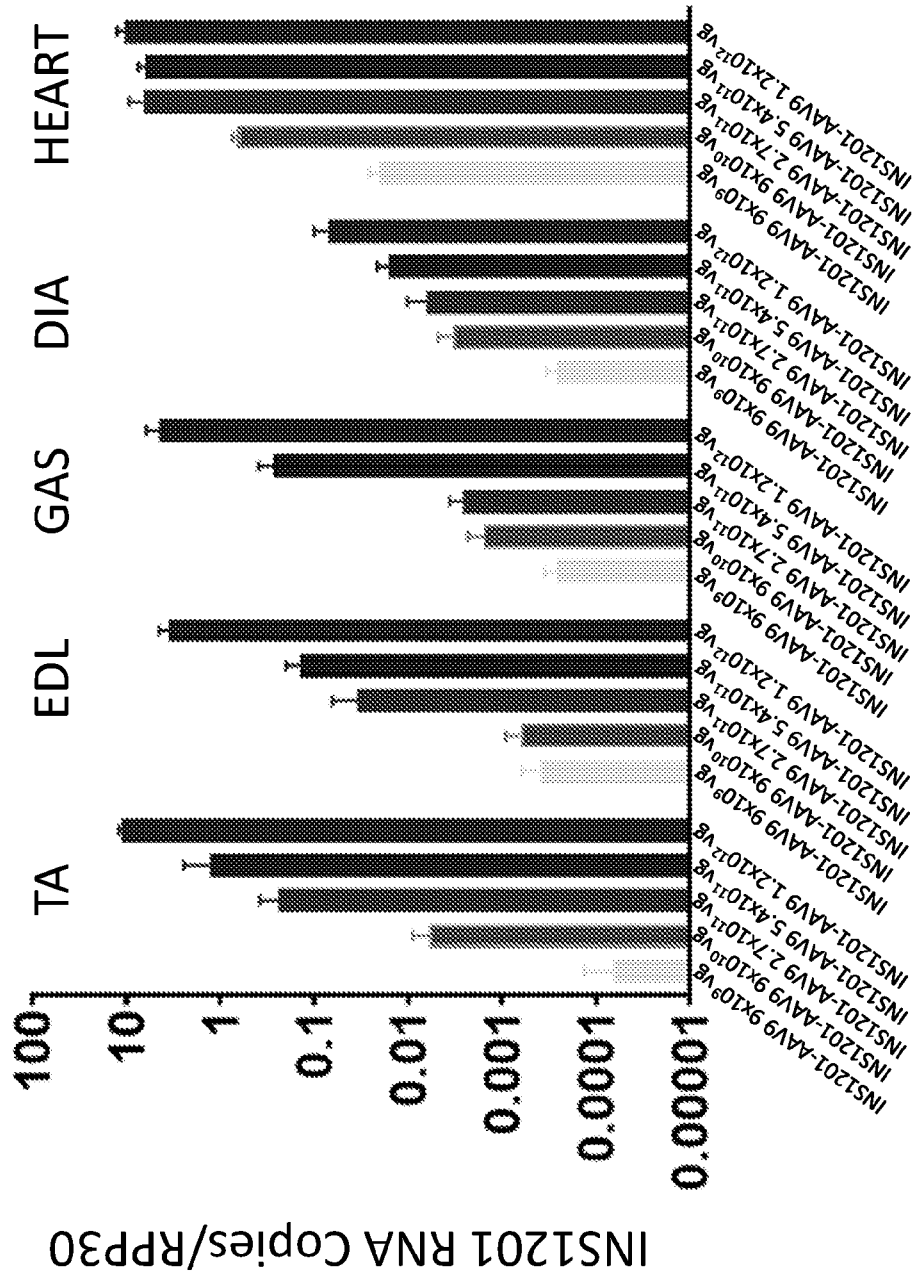

INS1201 RNA Copies/RPP30
100
10
1
0.1
0.01
0.001
0.0001
0.00001
TA
EDL
GAS
DIA
HEART
INS1201-AAV9 9x10⁹ vg
INS1201-AAV9 9x10¹⁰ vg
INS1201-AAV9 2.7x10¹¹ vg
INS1201-AAV9 5.4x10¹¹ vg
INS1201-AAV9 1.2x10¹² vg

FIG. 16

FIG. 17

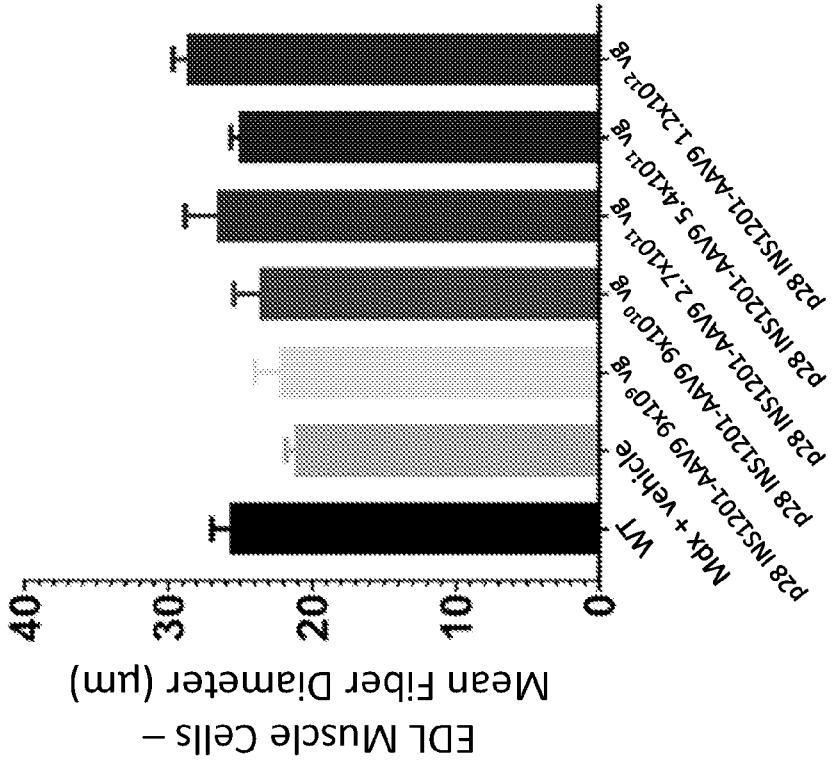

EDL Muscle Cells –
Mean Fiber Diameter (μm)
0
10
20
30
40
WT
Mdx + Vehicle
P28 INS1201-AAV9 9X10^9 vg
P28 INS1201-AAV9 9X10^10 vg
P28 INS1201-AAV9 2.7X10^11 vg
P28 INS1201-AAV9 5.4X10^11 vg
P28 INS1201-AAV9 1.2X10^12 vg

FIG. 18

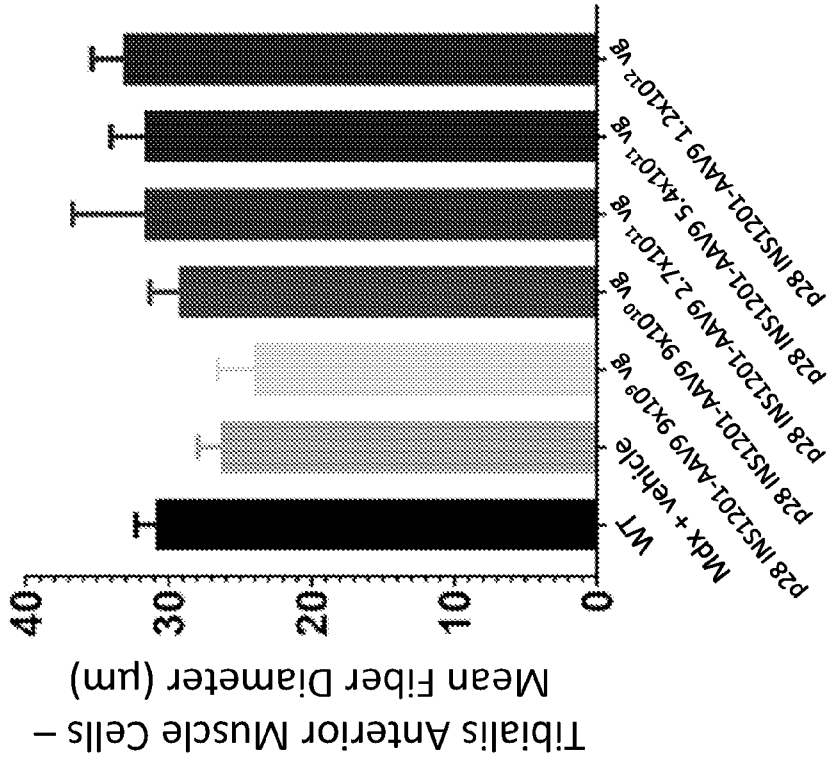

Tibialis Anterior Muscle Cells –
Mean Fiber Diameter (μm)
0
10
20
30
40
WT
Mdx + Vehicle
P28 INS1201-AAV9 9X10^9 vg
P28 INS1201-AAV9 9X10^10 vg
P28 INS1201-AAV9 2.7X10^11 vg
P28 INS1201-AAV9 5.4X10^11 vg
P28 INS1201-AAV9 1.2X10^12 vg

FIG. 19

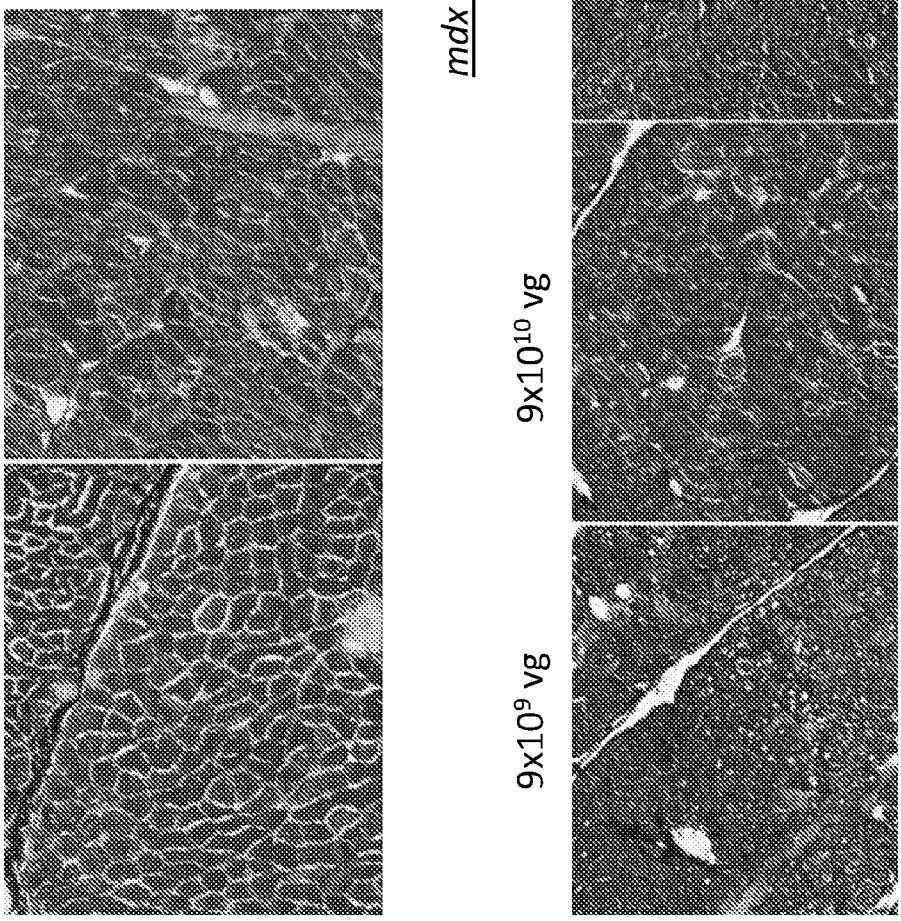
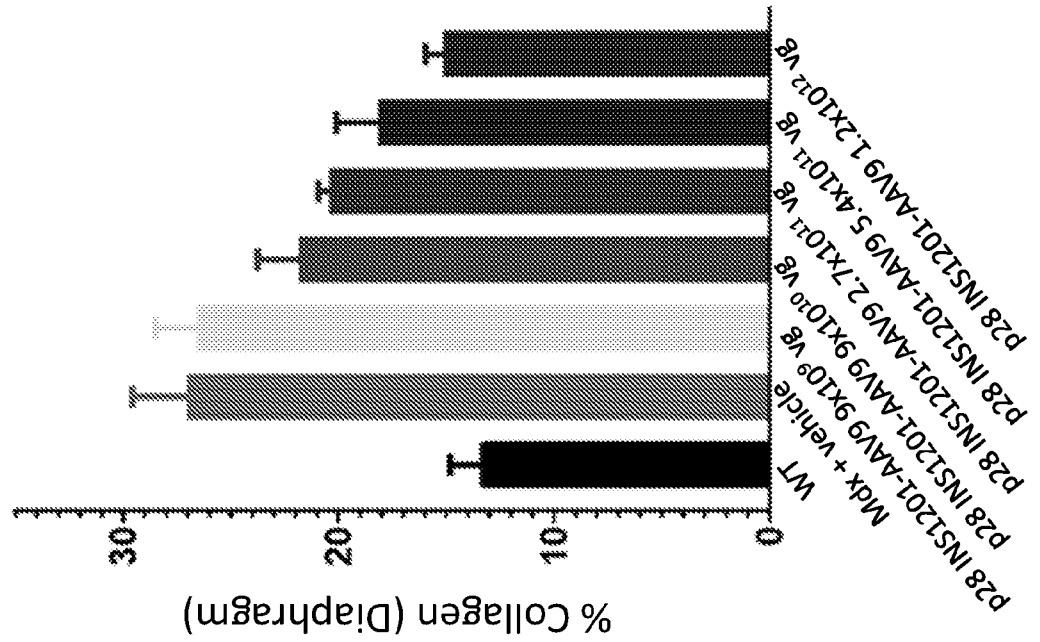

Controls
WT
mdx + vehicle
mdx + INS1201-AAV9
9x10^9 vg
9x10^10 vg
2.7x10^11 vg
5.4x10^11 vg
1.2x10^12 vg

FIG. 20


% Collagen (Diaphragm)
0
10
20
30
WT
Mdx + vehicle
p28 INS1201-AAV9 9X10^9 vg
p28 INS1201-AAV9 9X10^10 vg
p28 INS1201-AAV9 2.7X10^11 vg
p28 INS1201-AAV9 5.4X10^11 vg
p28 INS1201-AAV9 1.2X10^12 vg

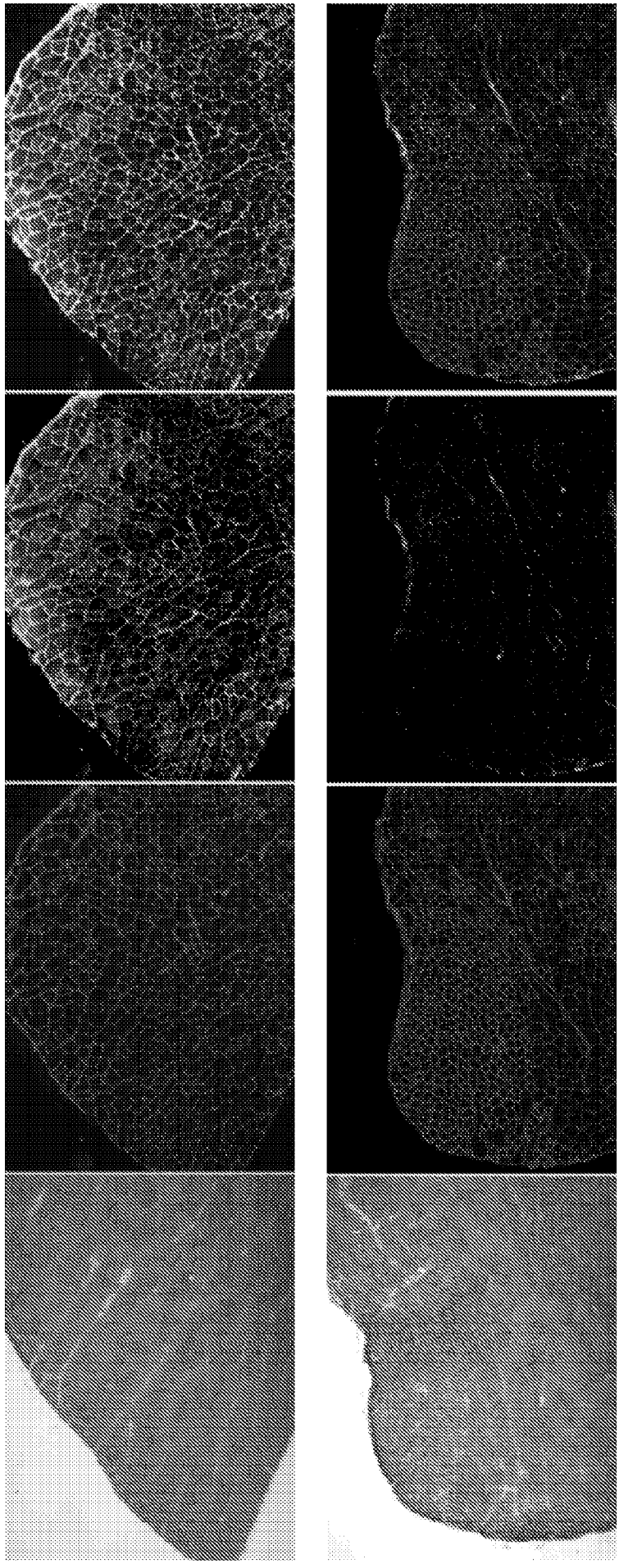


FIG. 21

Sequence Listing

1	**Sequence Listing Information**	
1-1	File Name	INMD_166_02WO_SeqList_ST26.xml
1-2	DTD Version	V1_3
1-3	Software Name	WIPO Sequence
1-4	Software Version	2.1.1
1-5	Production Date	2022-08-03
1-6	Original free text language code	en
1-7	Non English free text language code	
2	**General Information**	
2-1	Current application: IP Office	WO
2-2	Current application: Application number	
2-3	Current application: Filing date	
2-4	Current application: Applicant file reference	INMD-166/01WO
2-5	Earliest priority application: IP Office	US
2-6	Earliest priority application: Application number	63/239,881
2-7	Earliest priority application: Filing date	2021-09-01
2-8en	Applicant name	Insmed Incorporated
2-8	Applicant name: Name Latin	
2-9en	Inventor name	
2-9	Inventor name: Name Latin	
2-10en	Invention title	Adeno-Associated Virus Vectors and Methods of Use And Delivery Thereof
2-11	Sequence Total Quantity	13

3-1	**Sequences**	
3-1-1	Sequence Number [ID]	1
3-1-2	Molecule Type	DNA
3-1-3	Length	130
3-1-4	Features Location/	**source 1..130**
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		organism=synthetic construct
	NonEnglishQualifier Value	
3-1-5	Residues	ctgcgcgctc gctcgctcac tgaggccgcc cgggcaaagc ccgggcgtcg ggcgaccttt 60 ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact 120 aggggttcct 130
3-2	**Sequences**	
3-2-1	Sequence Number [ID]	2
3-2-2	Molecule Type	DNA
3-2-3	Length	771
3-2-4	Features Location/	**source 1..771**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-2-5	Residues	ccttcagatt aaaaataact gaggtaaggg cctgggtagg ggaggtggtg tgagacgctc 60 ctgtctctcc tctatctgcc catcggccct ttggggagga ggaatgtgcc caaggactaa 120 aaaaaggcca tggagccaga ggggcgaggg caacagacct ttcatgggca aaccttgggg 180 ccctgctgtc tagcatgccc cactacgggt ctaggctgcc catgtaagga ggcaaggcct 240 ggggacaccc gagatgcctg gttataatta acccagacat gtggctgccc ccccccccc 300 aacacctgct gcctctaaaa ataaccctgt ccctggtgga tcccctgcat gcgaagatct 360 tcgaacaagg ctgtggggga ctgagggcag gctgtaacag gcttgggggc cagggcttat 420 acgtgcctgg gactcccaaa gtattactgt tccatgttcc cggcgaaggg ccagctgtcc 480 cccgccagct agactcagca cttagtttag gaaccagtga gcaagtcagc ccttggggca 540 gcccatacaa ggccatgggg ctgggcaagc tgcacgcctg ggtccggggt gggcacggtg 600 cccgggcaac gagctgaaag ctcatctgct ctcaggggcc cctccctggg gacagcccct 660 cctggctagt cacaccctgt aggctcctct atataaccca ggggcacagg ggctgccctc 720 attctaccac cacctccaca gcacagacag acactcagga gccagccagc c 771
3-3	**Sequences**	
3-3-1	Sequence Number [ID]	3
3-3-2	Molecule Type	DNA
3-3-3	Length	270
3-3-4	Features Location/	**source 1..270**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-3-5	Residues	ccacgttctg cttcactctc cccatctccc ccccctcccc acccccaatt ttgtatttat 60 ttatttttta attattttgt gcagcgatgg gggcgggggg gggggggggg cgcgcgccag 120 gcggggcggg gcggggcgag gggcggggcg gggcgaggcg gagaggtgcg gcggcagcca 180 atcagagcgg cgcgctccga aagtttcctt ttatggcgag gcggcggcgg cggcggccct 240 ataaaaagcg aagcgcgcgg cgggcgggag 270
3-4	**Sequences**	
3-4-1	Sequence Number [ID]	4
3-4-2	Molecule Type	DNA
3-4-3	Length	98
3-4-4	Features Location/	**source 1..98**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-4-5	Residues	gtaagtttag tctttttttgt cttttatttc aggtcccgga tccggtggtg gtgcaaatca 60 aagaactgct cctcagtcga tgttgccttt acttctag 98
3-5	**Sequences**	
3-5-1	Sequence Number [ID]	5
3-5-2	Molecule Type	DNA
3-5-3	Length	3591
3-5-4	Features Location/	**source 1..3591**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-5-5	Residues	atgctttggt gggaagaagt agaggactgt tatgaaagag aagatgttca aaagaaaaca 60 ttcacaaaat gggtaaatgc acaattttct aagtttggga agcagcatat tgagaacctc 120

		ttcagtgacc tacaggatgg gaggcgcctc ctagacctcc tcgaaggcct gacagggcaa 180 aaactgccaa aagaaaaagg atccacaaga gttcatgccc tgaacaatgt caacaaggca 240 ctgcgggttt tgcagaacaa taatgttgat ttagtgaata ttggaagtac tgacatcgta 300 gatggaaatc ataaactgac tcttggtttg atttggaata taatcctcca ctggcaggtc 360 aaaaatgtaa tgaaaaatat catggctgga ttgcaacaaa ccaacagtga aaagattctc 420 ctgagctggg tccgacaatc aactcgtaat tatccacagg ttaatgtaat caacttcacc 480 accagctggt ctgatggcct ggctttgaat gctctcatcc atagtcatag gccagaccta 540 tttgactgga atagtgtggt ttgccagcag tcagccacac aacgactgga acatgcattc 600 aacatcgcca gatatcaatt aggcatagag aaactactcg atcctgaaga tgttgatacc 660 acctatccag ataagaagtc catcttaatg tacatcacat cactcttcca agttttgcct 720 caacaagtga gcattgaagc catccaggaa gtggaaatgt tgccaaggcc acctaaagtg 780 actaaagaag aacattttca gttacatcat caaatgcact attctcaaca gatcacggtc 840 agtctagcac agggatatga gagaacttct tcccctaagc ctcgattcaa gagctatgcc 900 tacacacagg ctgcttatgt caccacctct gaccctacac ggagcccatt tccttcacag 960 catttggaag ctcctgaaga caagtcattt ggcagttcat tgatggagag tgaagtaaac 1020 ctggaccgtt atcaaacagc tttagaagaa gtattatcgt ggcttctttc tgctgaggac 1080 acattgcaag cacaaggaga gatttctaat gatgtggaag tggtgaaaga ccagtttcat 1140 actcatgagg ggtacatgat ggatttgaca gcccatcagg gccgggttgg taatattcta 1200 caattgggaa gtaagctgat tggaacagga aaattatcag aagatgaaga aactgaagta 1260 caagagcaga tgaatctcct aaattcaaga tgggaatgcc tcagggtagc tagcatggaa 1320 aaacaaagca atttacatag agttttaatg gatctccaga atcagaaact gaaagagttg 1380 aatgactggc taacaaaaac agaagaaaga acaaggaaaa tggaggaaga gcctcttgga 1440 cctgatcttg aagacctaaa acgccaagta caacaacata aggtgcttca agaagatcta 1500 gaacaagaac aagtcagggt caattctctc actcacatgg tggtggtagt tgatgaatct 1560 agtggagatc acgcaactgc tgctttggaa gaacaactta aggtattggg agatcgatgg 1620 gcaaacatct gtagatggac agaagaccgc tgggttcttt tacaagacat ccttctcaaa 1680 tggcaacgtc ttactgaaga acagtgcctt tttagtgcat ggctttcaga aaaagaagat 1740 gcagtgaaca agattcacac aactggcttt aaagatcaaa atgaaatgtt atcaagtctt 1800 caaaaactgg ccgttttaaa agcggatcta gaaaagaaaa agcaatccat gggcaaactg 1860 tattcactca aacaagatct tctttcaaca ctgaagaata agtcagtgac ccagaagacg 1920 gaagcatggc tggataactt tgcccggtgt tgggataatt tagtccaaaa acttgaaaag 1980 agtacagcac agatttcaca ggctgtcacc accactcagc catcactaac acagacaact 2040 gtaatggaaa cagtaactac ggtgaccaca agggaacaga tcctggtaaa gcatgctcaa 2100 gaggaacttc caccaccacc tccccaaaag aagaggcaga ttactgtgga tcttgaaaga 2160 ctccaggaac ttcaagaggc cacggatgag ctggacctca agctgcgcca agctgaggtg 2220 atcaagggat cctggcagcc cgtgggcgat ctcctcattg actctctcca agatcacctc 2280 gagaaagtca aggcacttcg aggagaaatt gcgcctctga aagagaacgt gagccacgtc 2340 aatgaccttg ctcgccagct taccactttg ggcattcagc tctcaccgta taacctcagc 2400 actctggaag acctgaacac cagatggaag cttctgcagg tggccgtcga ggaccgagtc 2460 aggcagctgc atgaagccca cagggacttt ggtccagcat ctcagcactt tctttccacg 2520 tctgtccagg gtccctggga gagagccatc tcgccaaaca aagtgcccta ctatatcaac 2580 cacgagactc aaacaacttg ctgggaccat cccaaaatga cagagctcta ccagtcttta 2640 gctgacctga ataatgtcag attctcagct tataggactg ccatgaaact ccgaagactg 2700 cagaaggccc tttgcttgga tctcttgagc ctgtcagctg catgtgatgc cttggaccag 2760 cacaacctca agcaaaatga ccagcccatg gatatcctgc agattattaa ttgtttgacc 2820 actatttatg accgcctgga gcaagagcac aacaatttgg tcaacgtccc tctctgcgtg 2880 gatatgtgtc tgaactggct gctgaatgtt tatgatacgg gacgaacagg gaggatccgt 2940 gtcctgtctt ttaaaactgg catcatttcc ctgtgtaaag cacatttgga agacaagtac 3000 agataccttt tcaagcaagt ggcaagttca acaggatttt gtgaccagcg caggctgggc 3060 ctccttctgc atgattctat ccaaattcca agacagttgg gtgaagttgc atcctttggg 3120 ggcagtaaca ttgagccaag tgtccggagc tgcttccaat ttgctaataa taagccagag 3180 atcgaagcgg ccctcttcct agactggatg agactggaac cccagtccat ggtgtggctg 3240 cccgtcctgc acagagtggc tgctgcagaa actgccaagc atcaggccaa atgtaacatc 3300 tgcaaagagt gtccaatcat tggattcagg tacaggagtc taaagcactt taattatgac 3360 atctgccaaa gctgcttttt ttctggtcga gttgcaaaag gccataaaat gcactatccc 3420 atggtggaat attgcactcc gactacatca ggagaagatg ttcgagactt tgccaaggta 3480 ctaaaaaaca aatttcgaac caaaaggtat tttgcgaagc atccccgaat gggctacctg 3540 ccagtgcaga ctgtcttaga gggggacaac atggaaactg acacaattta g 3591
3-6	**Sequences**	
3-6-1	Sequence Number [ID]	6
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3-6-3	Length	82
3-6-4	Features Location/	**source 1..82**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct

	NonEnglishQualifier Value	
3-6-5	Residues	aacttgttta ttgcagctta taatggttac aaataaagca atagcatcac aaatttcaca 60 aataaagcat ttttttcact gc 82
3-7	**Sequences**	
3-7-1	Sequence Number [ID]	7
3-7-2	Molecule Type	DNA
3-7-3	Length	130
3-7-4	Features Location/	**source 1..130**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-7-5	Residues	aggaacccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctcactgagg 60 ccgggcgacc aaaggtcgcc cgacgcccgg gctttgcccg ggcggcctca gtgagcgagc 120 gagcgcgcag 130
3-8	**Sequences**	
3-8-1	Sequence Number [ID]	8
3-8-2	Molecule Type	DNA
3-8-3	Length	270
3-8-4	Features Location/	**source 1..270**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-8-5	Residues	ttctgagttc tctaaggtcc ctcactccca actcagaccc aagtcctgtc aattcccatt 60 cagtgtctga tctccttctt ctcaccttcc ccatcttcca tttgacccaa gcttcctgag 120 cactcctccc attccccttt ttggagtcct cctcctctcc cagaacccag taataagtgg 180 gctcctccct ggcctggacc cccatggtaa ccctataagg cgaggcagct gccatctgag 240 gcagggaggg gctggtgtgg gaggctaagg 270
3-9	**Sequences**	
3-9-1	Sequence Number [ID]	9
3-9-2	Molecule Type	DNA
3-9-3	Length	280
3-9-4	Features Location/	**source 1..280**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-9-5	Residues	cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt 60 gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca 120 atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc 180 aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta 240 catgacctta tgggactttc ctacttggca gtacatctac 280
3-10	**Sequences**	
3-10-1	Sequence Number [ID]	10
3-10-2	Molecule Type	DNA
3-10-3	Length	5283
3-10-4	Features Location/	**source 1..5283**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-10-5	Residues	ccttcagatt aaaaataact gaggtaaggg cctgggtagg ggaggtggtg tgagacgctc 60 ctgtctctcc tctatctgcc catcggccct ttggggagga ggaatgtgcc caaggactaa 120 aaaaaggcca tggagccaga ggggcgaggg caacagacct ttcatgggca aaccttgggg 180 ccctgctgtc tagcatgccc cactacgggt ctaggctgcc catgtaagga ggcaaggcct 240 ggggacaccc gagatgcctg gttataatta acccagacat gtggctgccc ccccccccc 300 aacacctgct gcctctaaaa ataaccctgt ccctggtgga tcccctgcat gcgaagatct 360 tcgaacaagg ctgtggggga ctgagggcag gctgtaacag gcttgggggc cagggcttat 420 acgtgcctgg gactcccaaa gtattactgt tccatgttcc cggcgaaggg ccagctgtcc 480 cccgccagct agactcagca cttagtttag gaaccagtga gcaagtcagc ccttggggca 540 gcccatacaa ggccatgggg ctgggcaagc tgcacgcctg ggtccggggt gggcacggtg 600 cccgggcaac gagctgaaag ctcatctgct ctcaggggcc cctccctggg gacagcccct 660 cctggctagt cacaccctgt aggctcctct atataaccca ggggcacagg ggctgccctc 720 attctaccac cacctccaca gccttcagat taaaaataac tgaggtaagg gcctgggtag 780 gggaggtggt gtgagacgct cctgtctctc ctctatctgc ccatcggccc tttggggagg 840 aggaatgtgc ccaaggacta aaaaaaggcc atggagccag aggggcgagg gcaacagacc 900 tttcatgggc aaaccttggg gccctgctgt ctagcatgcc ccactacggg tctaggctgc 960 ccatgtaagg aggcaaggcc tggggacacc cgagatgcct ggttataatt aacccagaca 1020

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3-11	**Sequences**	
3-11-1	Sequence Number [ID]	11
3-11-2	Molecule Type	DNA
3-11-3	Length	4714
3-11-4	Features Location/	**source 1..4714**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-11-5	Residues	ttctgagttc tctaaggtcc ctcactccca actcagaccc aagtcctgtc aattcccatt 60 cagtgtctga tctccttctt ctcaccttcc ccatcttcca tttgacccaa gcttcctgag 120 cactcctccc attccccttt ttggagtcct cctcctctcc cagaacccag taataagtgg 180 gctcctccct ggcctggacc cccatggtaa ccctataagg cgaggcagct gccatctgag 240 gcagggaggg gctggtgtgg gaggctaagg ccttcagatt aaaaataact gaggtaaggg 300 cctgggtagg ggaggtggtg tgagacgctc ctgtctctcc tctatctgcc catcggccct 360 ttggggagga ggaatgtgcc caaggactaa aaaaaggcca tggagccaga ggggcgaggg 420 caacagacct ttcatgggca aaccttgggg ccctgctgtc tagcatgccc cactacgggt 480 ctaggctgcc catgtaagga ggcaaggcct ggggacaccc gagatgcctg gttataatta 540 acccagacat gtggctgccc ccccccccc aacacctgct gcctctaaaa ataaccctgt 600 ccctggtgga tcccctgcat gcgaagatct tcgaacaagg ctgtggggga ctgagggcag 660 gctgtaacag gcttgggggc cagggcttat acgtgcctgg gactcccaaa gtattactgt 720 tccatgttcc cggcgaaggg ccagctgtcc cccgccagct agactcagca cttagtttag 780 gaaccagtga gcaagtcagc ccttggggca gcccatacaa ggccatgggg ctgggcaagc 840 tgcacgcctg ggtccggggt gggcacggtg cccgggcaac gagctgaaag ctcatctgct 900 ctcaggggcc cctccctggg gacagcccct cctggctagt cacaccctgt aggctcctct 960 atataaccca ggggcacagg ggctgccctc attctaccac cacctccaca gcacagacag 1020 acactcagga gccagccagc catgctttgg tgggaagaag tagaggactg ttatgaaaga 1080 gaagatgttc aaaagaaaac attcacaaaa tgggtaaatg cacaattttc taagtttggg 1140 aagcagcata ttgagaacct cttcagtgac ctacaggatg ggaggcgcct cctagacctc 1200 ctcgaaggcc tgacagggca aaaactgcca aaagaaaaag gatccacaag agttcatgcc 1260 ctgaacaatg tcaacaaggc actgcgggtt ttgcagaaca ataatgttga tttagtgaat 1320 attggaagta ctgacatcgt agatggaaat cataaactga ctcttggttt gatttggaat 1380 ataatcctcc actggcaggt caaaaatgta atgaaaaata tcatggctgg attgcaacaa 1440 accaacagtg aaaagattct cctgagctgg gtccgacaat caactcgtaa ttatccacag 1500 gttaatgtaa tcaacttcac caccagctgg tctgatggcc tggctttgaa tgctctcatc 1560 catagtcata ggccagacct atttgactgg aatagtgtgg tttgccagca gtcagccaca 1620 caacgactgg aacatgcatt caacatcgcc agatatcaat taggcataga gaaactactc 1680 gatcctgaag atgttgatac cacctatcca gataagaagt ccatcttaat gtacatcaca 1740 tcactcttcc aagttttgcc tcaacaagtg agcattgaag ccatccagga agtggaaatg 1800 ttgccaaggc cacctaaagt gactaaagaa gaacattttc agttacatca tcaaatgcac 1860 tattctcaac agatcacggt cagtctagca cagggatatg agagaacttc ttcccctaag 1920 cctcgattca agagctatgc ctacacacag gctgcttatg tcaccacctc tgaccctaca 1980 cggagcccat ttccttcaca gcatttggaa gctcctgaag acaagtcatt tggcagttca 2040 ttgatggaga gtgaagtaaa cctggaccgt tatcaaacag ctttagaaga agtattatcg 2100 tggcttcttt ctgctgagga cacattgcaa gcacaaggag agatttctaa tgatgtggaa 2160 gtggtgaaag accagtttca tactcatgag gggtacatga tggatttgac agcccatcag 2220 ggccgggttg gtaatattct acaattggga agtaagctga ttggaacagg aaaattatca 2280 gaagatgaag aaactgaagt acaagagcag atgaatctcc taaattcaag atgggaatgc 2340 ctcagggtag ctagcatgga aaaacaaagc aatttacata gagttttaat ggatctccag 2400 aatcagaaac tgaaagagtt gaatgactgg ctaacaaaaa cagaagaaag aacaaggaaa 2460 atggaggaag agcctcttgg acctgatctt gaagacctaa aacgccaagt acaacaacat 2520 aaggtgcttc aagaagatct agaacaagaa caagtcaggg tcaattctct cactcacatg 2580 gtggtggtag ttgatgaatc tagtggagat cacgcaactg ctgctttgga agaacaactt 2640 aaggtattgg gagatcgatg ggcaaacatc tgtagatgga cagaagaccg ctgggttctt 2700 ttacaagaca tccttctcaa atggcaacgt cttactgaag aacagtgcct ttttagtgca 2760 tggctttcag aaaaagaaga tgcagtgaac aagattcaca caactggctt taaagatcaa 2820 aatgaaatgt tatcaagtct tcaaaaactg gccgttttaa aagcggatct agaaaagaaa 2880 aagcaatcca tgggcaaact gtattcactc aaacaagatc ttctttcaac actgaagaat 2940 aagtcagtga cccagaagac ggaagcatgg ctggataact ttgcccggtg ttgggataat 3000

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3-12	**Sequences**	
3-12-1	Sequence Number [ID]	12
3-12-2	Molecule Type	DNA
3-12-3	Length	4730
3-12-4	Features Location/	**source 1..4730**
	Qualifiers	mol_type=other DNA
		organism=synthetic construct
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