

COMPOSITIONS AND METHODS FOR THE TREATMENT OF NEUROLOGICAL DISORDERS RELATED TO GLUCOSYLCERAMIDASE BETA 1 DEFICIENCY

RELATED APPLICATIONS

[01] This application claims the benefit of and priority to International Application No. PCT/US2023/061837, filed February 2, 2023, the contents of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

[02] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing file, entitled 14640-0070-01304_SL.xml, was created on January 31, 2024, and is 5,007,704 bytes in size. The information in electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

[03] Described herein are compositions and methods relating to polynucleotides, *e.g.* polynucleotides encoding glucosylceramidase beta 1 (GBA1) proteins and peptides for use in the treatment of Parkinson Disease (PD) and other GBA-related disorders, including Gaucher Disease, and Dementia with Lewy Bodies (collectively, “GBA-related disorders”). In some embodiments, compositions may be delivered in an adeno-associated viral (AAV) vector. In other embodiments, compositions described herein, may be used to treat a subject in need thereof, such as a human subject diagnosed with a GBA1-related disorder or other condition resulting from a deficiency in the quantity and/or function of GBA1 protein, or as a research tool in the study of diseases or conditions in cells or animal models of such disease or condition.

BACKGROUND

[04] Lysosomal acid glucosylceramidase, commonly called glucosylcerebrosidase or Gcase, a D-glucosyl-N-acylsphingosine glucohydrolase, is a lysosomal membrane protein important in glycolipid metabolism. The enzyme is encoded by the glucosylceramidase beta 1 (GBA1) gene (Ensembl Gene ID No. ENSG00000177628). This enzyme, together with Saposin A and Saposin C, catalyzes the hydrolysis of glucosylceramide to ceramide and glucose. See Vaccaro, Anna Maria, *et al.* Journal of Biological Chemistry 272.27 (1997): 16862-16867, the contents of which are incorporated herein by reference in their entirety.

[05] Mutations in GBA1 are known to cause disease in human subjects. Homozygous or compound heterozygous GBA1 mutations lead to Gaucher disease (“GD”). See Sardi, S. Pablo, Jesse M. Cedarbaum, and Patrik Brundin. Movement Disorders 33.5 (2018): 684-696, the

contents of which are herein incorporated by reference in their entirety. Gaucher disease is one of the most prevalent lysosomal storage disorders, with an estimated standardized birth incidence in the general population of between 0.4 to 5.8 individuals per 100,000. Heterozygous GBA1 mutations can lead to PD. Indeed, GBA1 mutations occur in 7-10% of total PD patients, making GBA1 mutations the most important genetic risk factor of PD. PD-GBA1 patients have reduced levels of the lysosomal enzyme beta-glucocerebrosidase (Gcase), which results in increased accumulations of glycosphingolipid glucosylceramide (GluCer), which in turn is correlated with exacerbated α -Synuclein aggregation and concomitant neurological symptoms. Gaucher disease and PD, as well as other lysosomal storage disorders or Lewy body diseases such as Dementia with Lewy Bodies, and related diseases, in some cases, share common etiology in the GBA1 gene. See Sidransky, E. and Lopez, G. Lancet Neurol. 2012 November; 11(11): 986–998, the contents of which are incorporated by reference in their entirety. Limited treatment options exist for such diseases.

[06] Consequently, there remains a long felt-need to develop pharmaceutical compositions and methods for the treatment of PD and other GBA1-related disorders and to ameliorate deficiencies of Gcase protein in patients afflicted with GBA1-related disorders.

SUMMARY

[07] The present disclosure addresses these challenges by providing AAV-based compositions and methods for treating Gcase deficiency in patients. Disclosed herein are compositions and methods directed to AAV-based gene delivery of Gcase to ameliorate loss-of-function and to improve intracellular lipid trafficking. The compositions and methods are useful to improve lysosomal glycolipid metabolism, and to slow, halt, or reverse neurodegenerative and other symptoms of PD and other GBA1-related disorders (e.g., dementia with Lewy Bodies (DLB), Gaucher disease (GD)) in a subject (e.g., a subject having a mutation in a GBA1 gene, e.g., a subject having a mutation in a GBA1 gene). Unless otherwise specified, GBA1 protein, GBA1 protein, and Gcase protein are synonymous terms and used interchangeably to refer to the protein encoded by the GBA1 gene.

[08] In some embodiments, the present disclosure provides nucleotide sequences encoding a wildtype GBA1 protein, wherein the GBA1 encoding nucleotide sequence comprises an altered GC-content, and/or a reduced number of CpG motifs (e.g., lacking all CpG motifs) as compared to a wildtype GBA1 encoding sequence (e.g., comprising the nucleotide sequence of SEQ ID NO: 1776 or 1777). In some embodiments, the GBA1-encoding nucleotide sequences surprisingly provide high GBA1 expression in the brain (e.g., the cortex, striatum, and

brainstem), high GBA1 activity (e.g., high glucosylceramide and glucosylsphingosine substrate clearance) in the brain, and reduced immunogenicity, e.g., as compared to a wildtype GBA1 encoding sequence (e.g., comprising the nucleotide sequence of SEQ ID NO: 1776 or 1777). In some embodiments, the GBA1-encoding nucleotide sequences described herein surprisingly provide reduced GBA1 expression in the dorsal root ganglion (DRG) while retaining high GBA1 activity in other areas of the brain (e.g., the brain stem), e.g., relative to a wildtype GBA1 encoding sequence (e.g., comprising the nucleotide sequence of SEQ ID NO: 1776 or 1777). In some embodiments, a nucleotide sequence encoding a wildtype GBA1 protein described herein can be administered to a subject having a GBA1-related disorder such as Parkinson's Disease.

[09] In some embodiments, the GBA1-encoding nucleotide sequence comprises SEQ ID NO: 2001 or a sequence at least 94% identical (e.g., at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) thereto. In some embodiments, the GBA1-encoding nucleotide sequence comprises SEQ ID NO: 2002 or a sequence at least 93% identical (e.g., at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) thereto.

[010] In some embodiments, the GBA1 encoding nucleotide sequence is comprised by an AAV viral genome comprising the nucleotide sequence of SEQ ID NO: 2006 or SEQ ID NO: 2007, or a sequence at least 97% identical (e.g., at least 97%, at least 98%, or at least 99%) thereto.

[011] In some embodiments, the GBA1 encoding nucleotide sequence (e.g., SEQ ID NO: 2001 or SEQ ID NO: 2002, or a sequence at least 94% identical (e.g., at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) thereto) comprises lower GC content than the nucleotide sequences of SEQ ID NOs: 1772, 1773, 1780, or 1781.

[012] In some embodiments, administration of a GBA1 encoding nucleotide sequence (e.g., a sequence comprising the nucleotide sequence of SEQ ID NO: 2001 or SEQ ID NO: 2002, or a sequence at least 94% identical (e.g., at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) thereto) to a subject results in higher GBA1 activity, e.g., higher glucosylceramide and glucosylsphingosine substrate reduction, in the brain of the subject as compared to administration of a sequence comprising the nucleotide sequences of SEQ ID NOs: 1772 or 1773.

[013] In some embodiments, administration of an AAV particle comprising a GBA1 encoding nucleotide sequence (e.g., a nucleotide sequence comprising SEQ ID NO: 2007, or a sequence at least 97% identical (e.g., at least 97%, at least 98%, or at least 99%) thereto) to a subject results in reduced GBA1 expression in the DRG as compared to administration of a

sequence comprising the nucleotide sequence of the nucleotide sequence of SEQ ID NO: 1772 or 1773, while the GBA1 activity in other brain regions (e.g., the brain stem) is not significantly reduced compared to administration of a sequence comprising the nucleotide sequence of the nucleotide sequence of SEQ ID NO: 1772 or 1773.

[014] In some embodiments, the present disclosure provides an isolated nucleic acid comprising a transgene encoding a GBA1 protein, wherein the nucleotide sequence encoding the GBA1 protein comprises a codon-optimized nucleotide sequence that is at least 93% identical (e.g., at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence of SEQ ID NO: 2002. In some embodiments, the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2002. In some embodiments, the isolated nucleic acid is or is comprised in a viral genome.

[015] In some embodiments, the present disclosure provides an isolated nucleic acid comprising a nucleotide sequence that encodes a β -glucocerebrosidase 1 (GBA1) protein and is at least 93% identical (e.g., at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence of SEQ ID NO: 2002. In some embodiments, the isolated nucleic acid encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2002. In some embodiments, the isolated nucleic acid is or is comprised in a viral genome.

[016] In some embodiments, the present disclosure provides an isolated nucleic acid comprising a transgene encoding a GBA1 protein, wherein the nucleotide sequence encoding the GBA1 protein comprises a codon-optimized nucleotide sequence that is at least 94% (e.g., at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) identical to the nucleotide sequence of SEQ ID NO: 2001. In some embodiments, the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2001. In some embodiments, the nucleotide sequence encoding the GBA1 protein consists of the nucleotide sequence of SEQ ID NO: 2001. In some embodiments, the isolated nucleic acid is or is comprised in a viral genome.

[017] In some embodiments, the present disclosure provides an isolated nucleic acid comprising a nucleotide sequence that encodes a β -glucocerebrosidase 1 (GBA1) protein and is at least 94% identical (e.g., at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence of SEQ ID NO: 2001. In some embodiments, the isolated nucleic acid encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2001. In some embodiments, the isolated nucleic acid encoding the

GBA1 protein consists of the nucleotide sequence of SEQ ID NO: 2001. In some embodiments, the isolated nucleic acid is or is comprised in a viral genome.

[018] In some embodiments, the disclosure provides an isolated nucleic acid comprising a transgene encoding a GBA1 protein and an enhancement element, wherein the encoded enhancement element comprises: a Saposin C polypeptide or functional fragment or variant thereof, optionally comprising the amino acid sequence of SEQ ID NO: 1789 or 1758, or an amino acid sequence at least 85% (e.g., at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) identical thereto; a cell penetrating peptide, optionally comprising the amino acid sequence of any of SEQ ID Nos: 1794, 1796, or 1798, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID Nos: 1794, 1796, or 1798; and/or a lysosomal targeting sequence, optionally comprising the amino acid sequence of any of SEQ ID Nos: 1800, 1802, 1804, 1806, or 1808, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID Nos: 1800, 1802, 1804, 1806, or 1808. In some embodiments, the isolated nucleic acid is or is comprised in a viral genome.

[019] In some embodiments, the present disclosure provides a recombinant viral genome comprising a nucleic acid comprising a transgene encoding a GBA1 protein, and further comprising a nucleotide sequence encoding a miR binding site that modulates, e.g., reduces, expression of the encoded GBA1 protein in a cell or tissue of the DRG, liver, hematopoietic lineage, or a combination thereof. In some embodiments, the encoded miR binding site comprises a miR183 binding site. In some embodiments, the viral genome encodes multiple miR binding sites, e.g., four miR183 binding sites. In some embodiments, the viral genome further encodes an enhancement element, e.g., an enhancement element described herein.

[020] In some embodiments, the present disclosure provides a recombinant viral genome comprising a promoter operably linked to a nucleic acid comprising a transgene encoding a GBA1 protein described herein. In some embodiments, the viral genome comprises an internal terminal repeat (ITR) sequence (e.g., an ITR region described herein), an enhancer (e.g., an enhancer described herein), an intron region (e.g., an intron region described herein), a Kozak sequence (e.g., a Kozak sequence described herein), an exon region (e.g., an exon region described herein), a nucleotide sequence encoding a miR binding site (e.g., a miR binding site described herein), and/or a poly A signal region (e.g., a poly A signal sequence described herein). In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID

NO: 2006 or 2007, or a nucleotide sequence at least 97% identical thereto (e.g., at least 97%, at least 98 or 99% identical thereto). In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 2006 or a nucleotide sequence at least 97% identical (e.g., at least 97%, at least 98 or 99% identical) thereto. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 2007 or a nucleotide sequence at least 97% identical (e.g., at least 97%, at least 98 or 99% identical) thereto .

[021] In some embodiments, the present disclosure provides a recombinant AAV particle comprising a capsid protein and a viral genome comprising a promoter (e.g., a promoter described herein) operably linked to a transgene encoding a GBA1 protein described herein. In some embodiments, the capsid protein comprises an AAV capsid protein. In some embodiments, the capsid protein comprises a VOY101 capsid protein, an AAV5 capsid protein, an AAV9 capsid protein, or a functional variant thereof. In some embodiments, the recombinant AAV particle is an isolated AAV particle.

[022] In some embodiments, the present disclosure provides a method of making a viral genome described herein. In some embodiments, the method of making a viral genome comprises providing a nucleic acid encoding a viral genome described herein and a backbone region suitable for replication of the viral genome in a cell, e.g., a bacterial cell (e.g., wherein the backbone region comprises one or both of a bacterial origin of replication and a selectable marker), and excising the viral from the backbone region, e.g., by cleaving the nucleic acid molecule at upstream and downstream of the viral genome.

[023] In some embodiments, the present disclosure provides a method of making a recombinant AAV particle. In some embodiments, the method of making a recombinant AAV particle comprises providing a host cell comprising a viral genome described herein and incubating the host cell under conditions suitable to enclose the viral genome in the AAV particle, e.g., a VOY101 capsid protein, thereby making the isolated AAV particle.

[024] In some embodiments, the present disclosure provides method of delivering a nucleic acid encoding GBA1 protein to a subject, the method comprising administering an effective amount of an AAV particle or a plurality of AAV particles, described herein, said AAV particle comprising a viral genome described herein, e.g., a viral genome comprising a nucleic acid comprising a transgene encoding a GBA1 protein described herein.

[025] In some embodiments, the present disclosure provides a method of treating a subject having or diagnosed with having a disease associated with GBA1 expression, a neurological disorder, or a neuromuscular disorder. In some embodiments, the method comprises administering an effective amount of an AAV particle or a plurality of AAV particles, described

herein, said AAV particle comprising a viral genome described herein, e.g., a viral genome comprising a nucleic acid comprising a transgene encoding a GBA1 protein described herein. In some embodiments, the disease associated with expression of GBA1 or the neurodegenerative or neuromuscular disorder comprises Parkinson's Disease (PD) (e.g., a PD associated with one or more mutations in a GBA1 gene), dementia with Lewy Bodies (DLB), Gaucher disease (GD) (e.g., Type 1 GD (GD1) or Type 3 GD (GD3)), Spinal muscular atrophy (SMA), Multiple System Atrophy (MSA), or Multiple sclerosis (MS).

[026] In some embodiments, the present disclosure provides AAV viral genomes comprising at least one inverted terminal repeat (ITR) and a payload region, wherein the payload region encodes one or more GBA1 proteins. In some embodiments, the AAV viral genome comprises a 5' ITR, a promoter, a payload region comprising a nucleotide sequence encoding a GBA1 protein, and a 3' ITR. The encoded protein may be a human (*Homo sapiens*) GBA1, a cynomolgus monkey (*Macaca fascicularis*) GBA1, or a rhesus monkey (*Macaca mulatta*) GBA1, a synthetic (non-naturally occurring) GBA1, or a derivative thereof, e.g., a variant that retains one or more function of a wild-type GBA1 protein. In some embodiments, the GBA1 may be at least partially humanized. In some embodiments, the encoded protein is a wild-type human GBA1 protein.

[027] The GCase of the present disclosure can be co-expressed with a saposin protein. In some embodiments, the transgene encoding the GCase includes a nucleotide sequence encoding the saposin protein. In some embodiments, the saposin protein is saposin A (SapA). In some embodiments, the saposin protein is saposin C (SapC).

[028] Viral genomes may be incorporated into an AAV particle, wherein the AAV particle comprises a viral genome and a capsid. In some embodiments, the capsid comprises a sequence as shown in Table 1.

[029] In some embodiments, the AAV particles described herein may be used in pharmaceutical compositions. The pharmaceutical compositions may be used to treat a disorder or condition associated with decreased GBA1 expression, activity, or protein levels. In some embodiments, the disorder or condition is a lysosomal lipid storage disorder. In some embodiments, the disorder or condition associated with decreased GBA1 protein levels is PD (e.g., a PD associated with one or more mutations in a GBA1 gene), Gaucher disease (e.g., Type 1 GD (e.g., non-neuronopathic GD (GD1)), Type 2 (e.g., acute neuronopathic GD (GD2)), or Type 3 GD (GD3)), or other GBA1-related disorder (e.g., dementia with Lewy Bodies (DLB)). In some embodiments, the disorder or condition associated with decreased GBA1 protein levels is PD. In some embodiments, the disorder or condition associated with decreased GBA1 protein

levels is GD. In some embodiments, the GD is GD1 or GD3. In some embodiments, the disorder or condition associated with decreased GBA1 protein level is DLB.

[030] In some embodiments, administration of AAV particles results in enhanced GBA1 expression in a target cell.

[031] In some aspects, the present disclosure provides methods of increasing GCase enzyme activity in patients using AAV-mediated gene transfer of an optimized GBA1 transgene cassette. The AAV-mediated gene transfer can be delivered to the CNS, and thereby decrease substrate glycosphingolipid glucosylceramide/GluCer levels and α -synuclein pathology, slowing or reversing disease pathogenesis in patients with GBA1-related disorders, including GBA1 patients with Parkinson's Disease (GBA1-PD), Gaucher disease (*e.g.*, Type 2 or 3 GD), and Dementia with Lewy body disease. In some embodiments, the methods involve intrastriatal (ISTR) or intracisternal (ICM) administration of AAV vectors packaging optimized GBA1 gene replacement transgene cassettes as described herein to achieve widespread, cell-autonomous transduction and cross-correction of a therapeutic GBA1 enzyme.

[032] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following enumerated embodiments.

Enumerated Embodiments

1. An isolated nucleic acid that encodes a β -glucocerebrosidase 1 (GBA1) protein and is at least 93% (*e.g.*, 93, 94, 95, 96, 97, 98, 99, or 100% identical) to the nucleotide sequence of SEQ ID NO: 2002.
2. The isolated nucleic acid of embodiment 1, wherein the nucleotide sequence encoding the GBA1 protein comprises a nucleotide sequence at least 93% identical to SEQ ID NO: 2002.
3. The isolated nucleic acid of embodiment 1 or 2, wherein the nucleotide sequence encoding the GBA1 protein comprises a nucleotide sequence at least 95% identical to SEQ ID NO: 2002.
4. The isolated nucleic acid of any one of embodiments 1-3, wherein the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2002.
5. The isolated nucleic acid of any one of embodiments 1-4, further comprising an enhancement element.

6. An isolated, e.g., recombinant, nucleic acid comprising a transgene encoding a β -glucocerebrosidase 1 (GBA1) protein and an enhancement element, wherein the encoded enhancement element comprises:
- (a) a Saposin C polypeptide or functional fragment or variant thereof, optionally comprising the amino acid sequence of SEQ ID NO: 1789 or 1758, or an amino acid sequence at least 85% (e.g., at least 90%, 92%, 95%, 97%, 98%, or 99%) identical thereto;
 - (b) a cell penetrating peptide, optionally comprising the amino acid sequence of any of SEQ ID NOs: 1794, 1796, or 1798, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NOs: 1794, 1796, or 1798; and/or
 - (c) a lysosomal targeting sequence, optionally comprising the amino acid sequence of any of SEQ ID NOs: 1800, 1802, 1804, 1806, or 1808, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NOs: 1800, 1802, 1804, 1806, or 1808.
7. A recombinant viral genome comprising a nucleic acid encoding a β -glucocerebrosidase 1 (GBA1) protein, further comprising a nucleotide sequence encoding a miR binding site that modulates, e.g., reduces, expression of the encoded GBA1 protein in a cell or tissue of the DRG, liver, hematopoietic lineage, or a combination thereof, wherein, optionally, the recombinant viral genome comprises the nucleic acid of any one of embodiments 1-6.
8. The viral genome of embodiment 7, wherein the nucleic acid further encodes an enhancement element.
9. The isolated nucleic acid of embodiment 5 or 6, or the viral genome of embodiment 8, wherein the encoded enhancement element comprises a Saposin C polypeptide or functional fragment or variant thereof.
10. The isolated nucleic acid of embodiment 5-6 or 9, or the viral genome of embodiment 8 or 9, wherein:
- (i) the encoded Saposin C polypeptide or functional fragment or variant thereof comprises the amino acid sequence of SEQ ID NO: 1789 or 1758, or an amino acid sequence at

least sequence at least 85% (e.g., at least 90%, 92%, 95%, 97%, 98%, or 99%) identical thereto; and/or

(ii) the nucleotide sequence encoding the encoded Saposin C polypeptide or functional fragment or variant thereof comprises the nucleotide sequence of SEQ ID NO: 1787 or 1791, or a nucleotide sequence at least 85% (e.g., at least 90%, 92%, 95%, 97%, 98%, or 99%) identical thereto.

11. The isolated nucleic acid of embodiment 5, or the viral genome of embodiment 8, wherein:

(i) the encoded enhancement element comprises the amino acid sequence of any of SEQ ID NOs: 1750, 1752, 1754, 1756-1758, 1784, or 1785, an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 1750, 1752, 1754, 1756-1758, 1784, or 1785, or an amino acid sequence at least 85% (e.g., at least 90%, 92%, 95%, 97%, 98%, or 99%) identical thereto; and/or

(ii) the nucleotide sequence encoding the enhancement element comprises the nucleotide sequence of any one of SEQ ID NOs: 1751, 1753, 1755, 1858, or 1859, or a nucleotide sequence at least 85% (e.g., at least 90%, 92%, 95%, 97%, 98%, or 99%) identical thereto.

12. The isolated nucleic acid of any one of embodiments 5-6 or 9-11, or the viral genome of embodiment 8-11, wherein the encoded enhancement element comprises a cell penetrating peptide.

13. The isolated nucleic acid of embodiment 6 or 12, or the viral genome of embodiment 12, wherein:

(i) the cell penetrating peptide comprises the amino acid sequence of any of SEQ ID NOs: 1794, 1796, or 1798, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NOs: 1794, 1796, or 1798;

(ii) the nucleotide sequence encoding the cell penetrating peptide comprises the nucleotide sequence of any of SEQ ID NOs: 1793, 1795, or 1797, or a nucleotide sequence at least 80% (e.g., 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99%) identical thereto.

14. The isolated nucleic acid of any one of embodiments 5-6 or 9-13, or the viral genome of any one of embodiments 8-13, wherein the encoded enhancement element comprises a lysosomal targeting sequence.

15. The isolated nucleic acid of embodiment 6 or 14, or the viral genome of any one of embodiment 14, wherein:

(i) the encoded lysosomal targeting sequence comprises the amino acid sequence of any of SEQ ID NOs: 1800, 1802, 1804, 1806, or 1808, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NOs: 1800, 1802, 1804, 1806, or 1808;

(ii) the nucleotide sequence encoding the lysosomal targeting sequence comprises the nucleotide sequence of any of SEQ ID NO: 1799, 1801, 1803, 1805, or 1807, or a nucleotide sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NOs: 1799, 1801, 1803, 1805, or 1807.

16. The isolated nucleic acid of any one of embodiments 5-6 or 9-15, or the viral genome of any one of embodiments 8-15, wherein the nucleic acid encodes at least 2, 3, 4 or more enhancement elements.

17. The isolated nucleic acid of any one of embodiments 5-6 or 9-16, or the viral genome of any one of embodiments 8-16, wherein the nucleic acid encodes two enhancement elements, wherein:

(i) the first enhancement element comprises a lysosomal targeting sequence, optionally wherein the lysosomal targeting sequence comprises the amino acid sequence of SEQ ID NO: 1802, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 1802; and

(ii) the second enhancement element comprises Saposin C polypeptide or functional fragment or variant thereof, optionally wherein the Saposin C polypeptide or functional fragment or variant thereof comprises the amino acid sequence of SEQ ID NO: 1789, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 1789.

18. The isolated nucleic acid or viral genome of embodiment 17, wherein the nucleic acid encoding the first enhancement element and the second enhancement element, comprises the nucleotide sequences of 1801 and 1787, a nucleotide sequence at least 85% (e.g., at least 90%, 92%, 95%, 97%, 98%, or 99%) identical to SEQ ID NOs: 1801 and 1787, or a nucleotide sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NOs: 1801 and 1787.

19. The isolated nucleic acid of any one of embodiments 5-6 or 9-17, or the viral genome of any one of embodiments 8-18, wherein the nucleic acid encodes a first enhancement element and a second enhancement element, wherein:

(i) the first enhancement element a cell penetrating peptide, optionally wherein the cell penetrating peptide comprises the amino acid sequence of SEQ ID NO: 1798, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 1798; and

(ii) the second enhancement element comprises a lysosomal targeting sequence, optionally wherein the lysosomal targeting sequence comprises the amino acid sequence of SEQ ID NO: 1802, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 1802.

20. The isolated nucleic acid or viral genome of embodiment 19, wherein the nucleic acid encoding the first enhancement element and the second enhancement element, comprises the nucleotide sequences of 1797 and 1801, a nucleotide sequence at least 85% (e.g., at least 90%, 92%, 95%, 97%, 98%, or 99%) identical to SEQ ID NOs: 1797 and 1801, or a nucleotide sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NOs: 1797 and 1801.

21. The isolated nucleic acid of any one of embodiments 5-6 or 9-20, or the viral genome of any one of embodiments 8-20, wherein the nucleic acid encodes a first enhancement element, a second enhancement element and a third enhancement element, wherein:

(i) the first enhancement element comprises a lysosomal targeting sequence, optionally wherein the lysosomal targeting sequence comprises the amino acid sequence of SEQ ID NO: 1802, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 1802;

(ii) the second enhancement element comprises a cell penetrating peptide, optionally wherein the cell penetrating peptide comprises the amino acid sequence of SEQ ID NO: 1798, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 1798; and

(iii) the third enhancement element comprises Saposin C polypeptide or functional fragment or variant thereof, optionally wherein the Saposin C polypeptide or functional fragment or variant thereof comprises amino acid sequence of SEQ ID NO: 1789, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 1789.

22. The isolated nucleic acid or viral genome of embodiment 21, wherein the nucleic acid encoding the first enhancement element, the second enhancement element, and the third enhancement element, comprises the nucleotide sequences of 1801, 1797, and 1787, a nucleotide sequence at least 85% (e.g., at least 90%, 92%, 95%, 97%, 98%, or 99%) identical to SEQ ID NOs: 1801, 1797, and 1787, or a nucleotide sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NOs: 1801, 1797, and 1787.

23. The isolated nucleic acid of any one of embodiments 1-6 or 9-22, or the viral genome of any one of embodiments 7-22, wherein the nucleic acid further encodes a linker.

24. The isolated nucleic acid of any one of embodiments 5-6 or 9-22, or the viral genome of any one of embodiments 8-22, wherein the encoded enhancement element and the encoded GBA1 protein are connected directly, e.g., without a linker.

25. The isolated nucleic acid of any one of embodiments 5-6 or 9-23, or the viral genome of any one of embodiments 8-23, wherein the encoded enhancement element and the encoded GBA1 protein are connected via the encoded linker.

26. The isolated nucleic acid or viral genome of embodiment 23 or 25, wherein:

(i) the encoded linker comprises the amino acid sequence of any of SEQ ID NOs: 1854, 1855, 1843, or 1845, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NOs: 1854, 1855, 1843, or 1845;

- (ii) the nucleotide sequence encoding the linker comprises the nucleotide sequence of any one of SEQ ID NOs: 1724, 1726, 1729, or 1730, or a nucleotide sequence having at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NOs: 1724, 1726, 1729, or 1730;
- (iii) the encoded linker comprises a furin cleavage site;
- (iv) the encoded linker comprises a T2A polypeptide;
- (v) the encoded linker comprises a (Gly4Ser)_n linker (SEQ ID NO: 1871), wherein n is 1-10, e.g., n is 3, 4, or 5; and/or
- (vi) the encoded linker comprises a (Gly4Ser)₃ linker (SEQ ID NO: 1845).

27. The isolated nucleic acid or the viral genome of any one of embodiments 23 or 25-26, wherein:

- (i) the encoded linker comprises the amino acid sequence of SEQ ID NO: 1854 and/or the amino acid sequence of SEQ ID NO: 1855, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions, relative to SEQ ID NO: 1854 and/or 1855; and/or
- (ii) the nucleotide sequence encoding the linker comprises the nucleotide sequence of SEQ ID NO: 1724 and/or the nucleotide sequence of SEQ ID NO: 1726, or a nucleotide sequence having at least one, two, or three but no more than four modifications, e.g., substitutions, relative to SEQ ID NO: 1724 and/or 1726.

28. The isolated nucleic acid of any one of embodiments 23 or 25-27, or the viral genome of any one of embodiments 23 or 25-26, wherein:

- (i) the encoded linker comprises the amino acid sequence of SEQ ID NO: 1845, or an amino acid sequence having at least one, two, or three but no more than four modifications, e.g., substitutions, relative to SEQ ID NO: 1845;
- (ii) the nucleotide sequence encoding the linker comprises the nucleotide sequence of SEQ ID NO: 1730, or a nucleotide sequence having at least one, two, or three but no more than four modifications, e.g., substitutions, relative to SEQ ID NO: 1730.

29. The isolated nucleic acid of any one of embodiments 5-6 or 9-28, or the viral genome of any one of embodiments 8-28, wherein the encoded GBA1 protein and the encoded enhancement element are expressed as a single polypeptide.

30. The isolated nucleic acid of any one of embodiments 5-6 or 9-28, or the viral genome of any one of embodiments 8-28, wherein the single polypeptide comprises a cleavage site present between the encoded GBA1 protein and the encoded enhancement element, optionally wherein the cleavage site is an T2A and/or a furin cleavage site.
31. The isolated nucleic acid of any one of embodiments 5-6 or 9-30, or the viral genome of any one of embodiments 8-30, wherein:
- (i) the nucleotide sequence encoding the enhancement element is located 5' relative to the nucleotide sequence encoding the GBA1 protein; and/or
 - (ii) the nucleotide sequence encoding the enhancement element is located 3' relative to the nucleotide sequence encoding the GBA1 protein.
32. The isolated nucleic acid of any one of embodiments 1-6 or 9-31, or the viral genome of any one of embodiments 7-31, wherein the encoded GBA1 protein comprises the amino acid sequence of SEQ ID NO: 1775, or an amino acid sequence at least 70% (e.g., at least 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99%) identical thereto.
33. The isolated nucleic acid of any one of embodiments 6 or 9-32, or the viral genome of any one of embodiments 7-32, wherein the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2002, or a nucleotide sequence at least 93% (e.g., at least 94%, 95%, 96%, 97%, 98%, or 99%) identical thereto.
34. The isolated nucleic acid of any one of embodiments 1-6 or 9-33, or the viral genome of any one of embodiments 7-33, wherein the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2002.
35. The isolated nucleic acid of any one of embodiments 1-6 or 9-34, or the viral genome of any one of embodiments 7-37, further encoding a signal sequence comprising any one of SEQ ID NOs: 1850-1853, 1856, 1857, or 2005.
36. The isolated nucleic acid or the viral genome of embodiment 35, wherein the encoded signal sequence comprises the amino acid sequence of SEQ ID NO: 2005.
37. The isolated nucleic acid or the viral genome of any one of embodiment 35 or 36, wherein the nucleotide sequence encoding the signal sequence is located:

- (i) 5' relative to the nucleotide sequence encoding the GBA1 protein; and/or
- (ii) 5' relative to the encoded enhancement element.

38. The isolated nucleic acid or the viral genome of embodiment 35 or 36, wherein the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2001 or a nucleotide sequence that is at least 97% (e.g., 97%, 98%, 99%, or 100% identical) thereto.

39. The isolated nucleic acid or the viral genome of embodiment 38, wherein the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2001.

40. The isolated nucleic acid or the viral genome of embodiment 38 or embodiment 39, wherein the nucleotide sequence encoding the GBA1 protein consists of the nucleotide sequence of SEQ ID NO: 2001.

39. An isolated nucleic acid or viral genome comprising a nucleotide sequence encoding a β -glucocerebrosidase 1 (GBA1) protein, wherein the GBA1-encoding nucleotide sequence comprises a nucleotide sequence encoding a signal sequence comprising the nucleotide sequence of SEQ ID NO: 2005, or a nucleotide sequence at least 85% (e.g., 85%, 88%, 90%, 92%, 95%, 96%, 97%, 98%, 99%, or 100% identical) thereto; and a nucleotide sequence encoding a GBA1 protein comprising the nucleotide sequence of SEQ ID NO: 2002, or a nucleotide sequence at least 93% (e.g., 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) thereto.

40. The isolated nucleic acid or viral genome of embodiment 39, wherein the nucleotide sequence encoding the signal sequence comprises the nucleotide sequence of SEQ ID NO: 2005 or a nucleotide sequence at least 99% identical thereto.

41. An isolated nucleic acid or viral genome comprising the nucleotide sequence of SEQ ID NO: 2001 or a nucleotide sequence at least 94% (e.g., 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) thereto, wherein the nucleotide sequence encodes a β -glucocerebrosidase 1 (GBA1) protein.

42. The isolated nucleic acid or viral genome of embodiment 41, wherein the nucleotide sequence encoding the GBA1 protein comprises or consists of the nucleotide sequence of SEQ ID NO: 2001.
43. An isolated, e.g., recombinant, viral genome comprising a promoter operably linked to the nucleic acid of any one of embodiments 1-6 or 9-42.
44. The viral genome of any one of embodiments 7-43, further comprising a promoter operably linked to the nucleic acid encoding the GBA1 protein.
45. The viral genome of any one of embodiments 7-44, which further comprises an enhancer.
46. The viral genome of embodiment 45, wherein the enhancer comprises a CMVie enhancer.
47. The viral genome of embodiment 45 or 46, wherein the enhancer comprises the nucleotide sequence of SEQ ID NO: 1831, or a nucleotide sequence at least 95% identical thereto.
48. The viral genome of any one of embodiments 43-47, wherein the promoter comprises a tissue specific promoter.
49. The viral genome of any one of embodiments 43-47, wherein the promoter comprises a ubiquitous promoter.
50. The viral genome of any one of embodiments 43-49, wherein the promoter comprises:
- (i) an EF-1a promoter, a chicken β -actin (CBA) promoter and/or its derivative CAG, a CMV immediate-early enhancer and/or promoter, a β glucuronidase (GUSB) promoter, a ubiquitin C (UBC) promoter, a neuron-specific enolase (NSE), a platelet-derived growth factor (PDGF) promoter, a platelet-derived growth factor B-chain (PDGF- β) promoter, an intercellular adhesion molecule 2 (ICAM-2) promoter, a synapsin (Syn) promoter, a methyl-CpG binding protein 2 (MeCP2) promoter, a Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) promoter, a metabotropic glutamate receptor 2 (mGluR2) promoter, a neurofilament light (NFL) or heavy (NFH) promoter, a β -globin minigene β 2 promoter, a preproenkephalin (PPE) promoter, an enkephalin (Enk) and excitatory amino acid transporter 2 (EAAT2), a glial fibrillary acidic protein (GFAP) promoter, a myelin basic protein (MBP) promoter, a cardiovascular promoter (e.g., α MHC, cTnT, and CMV-MLC2k), a liver promoter (e.g., hAAT,

TBG), a skeletal muscle promoter (e.g., desmin, MCK, C512) or a fragment, e.g., a truncation, or a functional variant thereof; and/or

(ii) the nucleotide sequence of any of SEQ ID NOs: 1832, 1833, 1834, 1835, 1836, 1839, 1840, or a nucleotide sequence at least 95% identical thereto.

51. The viral genome of any one of embodiments 43-47, wherein the promoter comprises a CB promoter or functional variant thereof.

52. The viral genome of embodiment 51, wherein the CB promoter or functional variant thereof comprises the nucleotide sequence of SEQ ID NO: 1834, or a nucleotide sequence at least 95% identical thereto.

53. The viral genome of any one of embodiments 51 or 52, wherein the promoter comprises a CMVie enhancer and a CB promoter.

54. The viral genome of embodiment 53, wherein the CMVie enhancer comprises the nucleotide sequence of SEQ ID NO: 1831, or a nucleotide sequence at least 95% identical thereto, and the CB promoter comprises the nucleotide sequence of SEQ ID NO: 1834, or a nucleotide sequence at least 95% identical thereto.

55. The viral genome of embodiment 50, wherein the promoter comprises an EF-1 α promoter or functional variant thereof.

56. The viral genome of embodiment 55, wherein the EF-1 α promoter or functional variant thereof comprises the nucleotide sequence of SEQ ID NO: 1839 or 1840, or a nucleotide sequence at least 95% identical thereto.

57. The viral genome of embodiment 55 or 56, wherein the EF-1 α promoter or functional variant thereof comprises an intron, e.g., an intron comprising the nucleotide sequence of positions 242-1180 of SEQ ID NO: 1839 or an intron comprising the nucleotide sequence of SEQ ID NO: 1841, or a nucleotide sequence at least 95% identical thereto.

58. The viral genome of any one of embodiments 55-57, wherein the EF-1 α promoter or functional variant thereof does not comprise an intron, e.g., an intron comprising the nucleotide

sequence of positions 242-1180 of SEQ ID NO: 1839 or an intron comprising the nucleotide sequence of SEQ ID NO: 1841, or a nucleotide sequence at least 95% identical thereto.

59. The viral genome of embodiment 50, wherein the promoter comprises a CBA promoter or functional variant thereof.

60. The viral genome of embodiment 59, wherein the CBA promoter functional variant thereof comprises the nucleotide sequence of SEQ ID NO: 1836, or a nucleotide sequence at least 95% identical thereto.

61. The viral genome of any one of embodiments 43-47, wherein the promoter comprises a CMVie enhancer, a CBA promoter or functional variant thereof, and an intron.

62. The viral genome of embodiment 61, wherein:

(i) the CMVie enhancer comprises the nucleotide sequence of SEQ ID NO: 1831, or a nucleotide sequence at least 95% identical thereto;

(ii) the CBA promoter or functional variant thereof comprises the nucleotide sequence of SEQ ID NO: 1836, or a nucleotide sequence at least 95% identical thereto; and

(iii) the intron comprises the nucleotide sequence of SEQ ID NO: 1837, or a nucleotide sequence at least 95% identical thereto.

63. The viral genome of embodiment 50, wherein the promoter comprises a CAG promoter region.

64. The viral genome of any one of embodiments 43-47 or 63, wherein the promoter comprises a CAG promoter region comprising:

(i) a CMVie enhancer, a CBA promoter or functional variant thereof, and an intron; and/or

(ii) the nucleotide sequence of SEQ ID NO: 1835, or a nucleotide sequence at least 95% identical thereto.

65. The viral genome of any one of embodiments 43-47, wherein the promoter comprises a CMV promoter or functional variant thereof.

66. The viral genome of embodiment 67, wherein the CMV promoter or functional variant thereof comprises the nucleotide sequence of SEQ ID NO: 1832, or a nucleotide sequence at least 95% identical thereto.

67. The viral genome of any one of embodiments 43-47, wherein the promoter comprises a CMVie enhancer and a CMV promoter or functional variant thereof, optionally wherein the CMVie enhancer comprises the nucleotide sequence of SEQ ID NO: 1831, or a nucleotide sequence at least 95% identical thereto, and the CMV promoter or functional variant thereof comprises the nucleotide sequence of SEQ ID NO: 1832, or a nucleotide sequence at least 95% identical thereto.

68. The viral genome of any one of embodiments 43-47, wherein the promoter comprises a CMV promoter region.

69. The viral genome of embodiment 68, wherein the CMV promoter region comprises:
(i) a CMVie enhancer and a CMV promoter or functional variant thereof;
(ii) the nucleotide sequence of SEQ ID NO: 1833, or a nucleotide sequence at least 95% identical thereto.

70. The viral genome of any one of embodiments 7-69, which further comprises an inverted terminal repeat (ITR) sequence.

71. The viral genome of embodiment 70, wherein the ITR sequence is positioned 5' relative to the nucleic acid encoding the GBA1 protein.

72. The viral genome of embodiment 70 or 71, wherein the ITR sequence is positioned 3' relative to the nucleic acid encoding the GBA1 protein.

73. The viral genome of any one of embodiments 7-72, which comprises an ITR positioned 5' relative to the nucleic acid encoding the GBA1 protein and an ITR positioned 3' relative to the nucleic acid encoding the GBA1 protein.

74. The viral genome of any one of embodiments 70-73, wherein the ITR comprises a nucleic acid sequence of SEQ ID NO: 1829, 1830, or 1862, or a nucleotide sequence at least 95% identical thereto.

75. The viral genome of any one of embodiments 70-74, wherein the ITR comprises the nucleotide sequence of SEQ ID NO: 1860 and/or 1861, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1860 and/or 1861.

76. The viral genome of any one of embodiments 70-75, wherein the ITR is positioned 5' relative to the nucleic acid encoding the GBA1 protein and comprises the nucleotide sequence of SEQ ID NO: 1860 and/or 1861, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1860 or 1861.

77. The viral genome of any one of embodiments 70-76, wherein the ITR is positioned 3' relative to the nucleic acid encoding the GBA1 protein and comprises the nucleotide sequence of SEQ ID NO: 1860 or 1861, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1860 and/or 1861.

78. The viral genome of any one of embodiments 70-77, wherein:

(i) the ITR positioned 5' relative to the nucleic acid encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 1829, or a nucleotide sequence at least 95% identical thereto; and/or

(ii) the ITR positioned 3' relative to the nucleic acid encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 1830, or a nucleotide sequence at least 95% identical thereto.

79. The viral genome of any one of embodiments 70-78, which further comprises a polyadenylation (polyA) signal region.

80. The viral genome of embodiment 79, wherein the polyA signal region comprises the nucleotide sequence of SEQ ID NO: 1846, or a nucleotide sequence at least 95% identical thereto.

81. The viral genome of any one of embodiments 7-80, which further comprises an intron region.
82. The viral genome of embodiment 81, wherein the intron comprises a beta-globin intron.
83. The viral genome of embodiment 81 or 82, wherein the intron comprises the nucleotide sequence of SEQ ID NO: 1842, or a nucleotide sequence at least 95% identical thereto.
84. The viral genome of any one of embodiments 7-83, which further comprises an exon region, e.g., at least one, two, or three exon regions.
85. The viral genome of any one of embodiments 7-84, which further comprises a Kozak sequence.
86. The viral genome of any one of embodiments 7-85, which further comprises a nucleotide sequence encoding a miRNA (miR) binding site, e.g., a miR binding site that modulates, e.g., reduces, expression of the GBA1 protein encoded by the viral genome in a cell or tissue where the corresponding miRNA is expressed.
87. The viral genome of embodiment 86, wherein the encoded miR binding site is fully or partially complementary to a miRNA expressed in a cell or tissue of the DRG, liver, hematopoietic, or a combination thereof.
88. The viral genome of embodiment 87, wherein the encoded miR binding site modulates, e.g., reduces, expression of the encoded GBA1 protein in a cell or tissue of the DRG, liver, hematopoietic lineage, or a combination thereof.
89. The viral genome of any one of embodiments 86-88, which comprises at least 1, 2, 3, 4, or 5 copies of the nucleotide sequence encoding the miR binding site.
90. The viral genome of any one of embodiments 86-89, which comprises at least 4 copies of the nucleotide sequence encoding the miR binding site, optionally wherein all four copies encode the same miR binding site.

91. The viral genome of embodiment 90, wherein the 4 copies of the nucleic acid encoding the miR binding site are continuous.

92. The viral genome of embodiment 90, wherein the 4 copies of the nucleic acid encoding the miR binding site are separated by a spacer.

93. The viral genome of embodiment 92, wherein the spacer comprises the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848.

94. The viral genome of any one of embodiments 86-93, wherein the encoded miR binding site comprises a miR183 binding site, a miR122 binding site, a miR-142-3p, or a combination thereof, optionally wherein:

(i) the encoded miR183 binding site comprises the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity, e.g., 100% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847;

(ii) the encoded miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1865, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity, e.g., 100% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1865; and/or

(iii) the encoded miR-142-3p binding site comprises the nucleotide sequence of SEQ ID NO: 1869, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity, e.g., 100% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1869.

95. The viral genome of any one of embodiments 86-94, wherein the viral genome comprises a nucleotide sequence encoding a miR183 binding site.

96. The viral genome of embodiment 95, wherein the viral genome encodes at least 1-5 copies, e.g., 4 copies of a miR183 binding site.

97. The viral genome of embodiment 96, wherein each copy is continuous .
98. The viral genome of embodiment 96, wherein each copy is separated by a spacer.
99. The viral genome of any one of embodiments 95-98, wherein the encoded miR183 binding site comprises the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity, e.g., 100% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847.
100. The viral genome of embodiment 98, wherein the viral genome comprises:
- (i) a first encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity, e.g., 100% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847;
 - (ii) a first spacer sequence comprising the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848;
 - (iii) a second encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity, e.g., 100% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847;
 - (iv) a second spacer sequence comprising the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848;
 - (v) a third encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity, e.g., 100% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847;

(vi) a third spacer sequence comprising the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848; and

(vii) a fourth encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity, e.g., 100% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847.

101. The viral genome of any one of embodiments 7-100, which comprises a miR183 binding site series, which comprises four copies of a miR183 binding site, wherein each copy of the miR binding site in the series is separated by a spacer.

102. The viral genome of embodiment 101, wherein the encoded miR183 binding site series comprises the nucleotide sequence of SEQ ID NO: 1849, or a nucleotide sequence at least 95% identical thereto.

103. The viral genome of embodiment 102, wherein the encoded miR183 binding site series comprises or consists of the nucleotide sequence of SEQ ID NO: 1849.

104. The viral genome of any one of embodiments 7-103, which is self-complementary.

105. The viral genome of any one of embodiments 7-103, which is single-stranded.

106. A recombinant viral genome comprising, in 5' to 3' order:

(i) a 5' adeno-associated (AAV) ITR, optionally wherein the 5' AAV ITR comprises the nucleotide sequence of SEQ ID NO: 1829, or a nucleotide sequence at least 95% identical thereto;

(ii) a CMVie enhancer, optionally wherein the CMVie enhancer comprises the nucleotide sequence of SEQ ID NO: 1831, or a nucleotide sequence at least 95% identical thereto;

(iii) a CB promoter or functional variant thereof, optionally wherein the CB promoter or functional variant thereof comprises the nucleotide sequence of SEQ ID NO: 1834, or a nucleotide sequence at least 95% identical thereto;

- (iv) an intron, optionally wherein the intron comprises the nucleotide sequence of SEQ ID NO: 1842, or a nucleotide sequence at least 95% identical thereto;
- (v) a nucleotide sequence encoding a signal sequence, optionally wherein the nucleotide sequence encoding the signal sequence comprises the nucleotide sequence of SEQ ID NO: 2005, or a nucleotide sequence at least 95% identical thereto;
- (vi) a nucleotide sequence encoding a GBA1 protein, wherein the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2002 or a nucleotide sequence at least 93% identical to the nucleotide sequence of SEQ ID NO: 2002;
- (vii) a polyA signal region, optionally wherein the polyA signal region comprises the nucleotide sequence of SEQ ID NO: 1846, or a nucleotide sequence at least 95% identical thereto; and
- (viii) a 3' AAV ITR, optionally wherein the 3' AAV ITR comprises the nucleotide sequence of SEQ ID NO: 1830, or a nucleotide sequence at least 95% identical thereto.

107. The recombinant viral genome of embodiment 106, wherein:

- (i) the 5' AAV ITR comprises the nucleotide sequence of SEQ ID NO: 1829, or a nucleotide sequence at least 95% identical thereto;
- (ii) the CMVie enhancer comprises the nucleotide sequence of SEQ ID NO: 1831, or a nucleotide sequence at least 95% identical thereto;
- (iii) the CB promoter or functional variant thereof comprises the nucleotide sequence of SEQ ID NO: 1834, or a nucleotide sequence at least 95% identical thereto;
- (iv) the nucleotide sequence of SEQ ID NO: 1842, or a nucleotide sequence at least 95% identical thereto;
- (v) the nucleotide sequence encoding the signal sequence comprises the nucleotide sequence of SEQ ID NO: 2005, or a nucleotide sequence at least 95% identical thereto;
- (vi) the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2002 or a nucleotide sequence at least 93% identical to the nucleotide sequence of SEQ ID NO: 2002;
- (vii) the polyA signal region comprises the nucleotide sequence of SEQ ID NO: 1846, or a nucleotide sequence at least 95% identical thereto; and
- (viii) the 3' AAV ITR comprises the nucleotide sequence of SEQ ID NO: 1830, or a nucleotide sequence at least 95% identical thereto

108. The recombinant viral genome of embodiment 106 or 107, wherein:

- (i) the 5' AAV ITR comprises the nucleotide sequence of SEQ ID NO: 1829;
 - (ii) the CMVie enhancer comprises the nucleotide sequence of SEQ ID NO: 1831;
 - (iii) the CB promoter or functional variant thereof comprises the nucleotide sequence of SEQ ID NO: 1834;
 - (iv) the intron comprises the nucleotide sequence of SEQ ID NO: 1842;
 - (v) the nucleotide sequence encoding the signal sequence comprises the nucleotide sequence of SEQ ID NO: 2005;
 - (vi) the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2002;
 - (vii) the polyA signal region comprises the nucleotide sequence of SEQ ID NO: 1846;
- and
- (viii) the 3' AAV ITR comprises the nucleotide sequence of SEQ ID NO: 1830.

109. The recombinant viral genome of embodiment 108, comprising the nucleotide sequence of SEQ ID NO: 2006, or a nucleotide sequence at least 97% identical thereto.

110. The recombinant viral genome of embodiment 108 or 109, comprising the nucleotide sequence of SEQ ID NO: 2006.

111. The recombinant viral genome of embodiment 108 or 109, consisting of the nucleotide sequence of SEQ ID NO: 2006.

112. A recombinant viral genome comprising in 5' to 3' order:

- (i) a 5' adeno-associated (AAV) ITR, optionally wherein the 5' AAV ITR comprises the nucleotide sequence of SEQ ID NO: 1829, or a nucleotide sequence at least 95% identical thereto;
- (ii) a CMVie enhancer, optionally wherein the CMVie enhancer comprises the nucleotide sequence of SEQ ID NO: 1831, or a nucleotide sequence at least 95% identical thereto;
- (iii) a CB promoter or functional variant thereof, optionally wherein the CB promoter or functional variant thereof comprises the nucleotide sequence of SEQ ID NO: 1834, or a nucleotide sequence at least 95% identical thereto;
- (iv) an intron, optionally wherein the intron comprises the nucleotide sequence of SEQ ID NO: 1842, or a nucleotide sequence at least 95% identical thereto;

(v) a nucleotide sequence encoding a signal sequence, optionally wherein the nucleotide sequence encoding the signal sequence comprises the nucleotide sequence of SEQ ID NO: 2005, or a nucleotide sequence at least 95% identical thereto;

(vi) a nucleotide sequence encoding a GBA1 protein, optionally wherein the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2002 or a nucleotide sequence at least 93% identical to the nucleotide sequence of SEQ ID NO: 2002;

(vii) a miR183 binding site series;

(viii) a polyA signal region, optionally wherein the polyA signal region comprises the nucleotide sequence of SEQ ID NO: 1846, or a nucleotide sequence at least 95% identical thereto; and

(ix) a 3' AAV ITR, optionally wherein the 3' AAV ITR comprises the nucleotide sequence of SEQ ID NO: 1830, or a nucleotide sequence at least 95% identical thereto;

wherein the miR183 binding site series comprises:

(a) a miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847;

(b) a spacer sequence comprising the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848;

(c) a miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847;

(d) a spacer sequence comprising the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848;

(e) a miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847;

(f) a spacer sequence comprising the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848;

(g) a miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847.

113. The recombinant viral genome of embodiment 112,

(i) the 5' AAV ITR comprises the nucleotide sequence of SEQ ID NO: 1829, or a nucleotide sequence at least 95% identical thereto;

(ii) the CMV_{ie} enhancer comprises the nucleotide sequence of SEQ ID NO: 1831, or a nucleotide sequence at least 95% identical thereto;

(iii) the CB promoter or functional variant thereof comprises the nucleotide sequence of SEQ ID NO: 1834, or a nucleotide sequence at least 95% identical thereto;

(iv) the nucleotide sequence of SEQ ID NO: 1842, or a nucleotide sequence at least 95% identical thereto;

(v) the nucleotide sequence encoding the signal sequence comprises the nucleotide sequence of SEQ ID NO: 2005, or a nucleotide sequence at least 95% identical thereto;

(vi) the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2002 or a nucleotide sequence at least 93% identical to the nucleotide sequence of SEQ ID NO: 2002;

(vii) the miR183 binding site series comprises:

(a) a miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847;

(b) a spacer sequence comprising the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848;

(c) a miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847;

(d) a spacer sequence comprising the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848;

(e) a miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847;

(f) a spacer sequence comprising the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848;

- (g) a miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 1847, or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, but no more than ten modifications of SEQ ID NO: 1847;
- (viii) the polyA signal region comprises the nucleotide sequence of SEQ ID NO: 1846, or a nucleotide sequence at least 95% identical thereto; and
- (ix) the 3' AAV ITR comprises the nucleotide sequence of SEQ ID NO: 1830, or a nucleotide sequence at least 95% identical thereto.

114. The recombinant viral genome of embodiment 106, 107, 112, or 113, wherein the nucleotide sequence encoding a GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2002, or a nucleotide sequence at least 95% identical thereto.

115. The recombinant viral genome of embodiment 113 or 114, wherein:

- (i) the 5' AAV ITR comprises the nucleotide sequence of SEQ ID NO: 1829;
 - (ii) the CMVie enhancer comprises the nucleotide sequence of SEQ ID NO: 1831;
 - (iii) the CB promoter or functional variant thereof comprises the nucleotide sequence of SEQ ID NO: 1834;
 - (iv) the intron comprises the nucleotide sequence of SEQ ID NO: 1842;
 - (v) the nucleotide sequence encoding the signal sequence comprises the nucleotide sequence of SEQ ID NO: 2005;
 - (vi) the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2002;
 - (vii) the miR183 binding site series comprises the nucleotide sequence of SEQ ID NO: 1849;
 - (viii) the polyA signal region comprises the nucleotide sequence of SEQ ID NO: 1846;
- and
- (ix) the 3' AAV ITR comprises the nucleotide sequence of SEQ ID NO: 1830.

116. The recombinant viral genome of embodiment 115, comprising the nucleotide sequence of SEQ ID NO: 2007, or a nucleotide sequence at least 97% identical thereto.

117. The recombinant viral genome of embodiment 115 or embodiment 116, comprising or consisting of the nucleotide sequence of SEQ ID NO: 2007.

118. The recombinant viral genome of any one of embodiments 7-117, which further comprises a nucleic acid encoding a capsid protein, wherein the capsid protein comprises a VP1 polypeptide, a VP2 polypeptide, and/or a VP3 polypeptide.

119. The recombinant viral genome of embodiment 118, wherein the VP1 polypeptide, the VP2 polypeptide, and/or the VP3 polypeptide are encoded by at least one Cap gene.

120. The viral genome of any one of embodiments 7-119, which further comprises a nucleic acid encoding a Rep protein, wherein the Rep protein comprises a Rep78 protein, a Rep68, Rep52 protein, and/or a Rep40 protein.

121. The viral genome of embodiment 120, wherein the Rep78 protein, the Rep68 protein, the Rep52 protein, and/or the Rep40 protein are encoded by at least one Rep gene.

122. An AAV particle comprising:

- (i) a capsid protein; and
- (ii) the viral genome of any one of embodiments 7-121.

123. The AAV particle of embodiment 122, wherein:

- (i) the capsid protein comprises the amino acid sequence of SEQ ID NO: 138, or an amino acid sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto;
- (ii) the capsid protein comprises an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of the amino acid sequence of SEQ ID NO: 138;
- (iii) the capsid protein comprises the amino acid sequence of SEQ ID NO: 11, or an amino acid sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto;
- (iv) the capsid protein comprises an amino acid sequence having at least one, two or three modifications but not more than 30, 20 or 10 modifications of the amino acid sequence of SEQ ID NO: 11;
- (v) the capsid protein comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 137, or a sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto; and/or

(vi) the nucleotide sequence encoding the capsid protein comprises the nucleotide sequence of SEQ ID NO: 137, or a sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

124. The AAV particle of embodiment 122 or 123, wherein the capsid protein comprises:

(i) an amino acid substitution at position K449, e.g., a K449R substitution, numbered according to SEQ ID NO:138;

(ii) an insert comprising the amino acid sequence of TLAVPFK (SEQ ID NO: 1262), optionally wherein the insert is present immediately subsequent to position 588, relative to a reference sequence numbered according to SEQ ID NO:138;

(iii) an amino acid other than "A" at position 587 and/or an amino acid other than "Q" at position 588, numbered according to SEQ ID NO: 138;

(iv) the amino acid substitution of A587D and/or Q588G, numbered according to SEQ ID NO:138.

125. The AAV particle of any one of embodiments 122-124, wherein the capsid protein comprises (i) the amino acid substitution of K449R numbered according to SEQ ID NO:138; and (ii) an insert comprising the amino acid sequence of TLAVPFK (SEQ ID NO: 1262), optionally wherein the insert is present immediately subsequent to position 588 of SEQ ID NO:138.

126. The AAV particle of any one of embodiments 122-124, wherein the capsid protein comprises (i) the amino acid substitution of K449R numbered according to SEQ ID NO:138; (ii) an insert comprising the amino acid sequence of TLAVPFK (SEQ ID NO: 1262), optionally wherein the insert is present immediately subsequent to position 588, relative to a reference sequence numbered according to SEQ ID NO:138; and (iii) the amino acid substitutions of A587D and Q588G, numbered according to SEQ ID NO:138.

127. The AAV particle of any one of embodiments 122-124, wherein the capsid protein comprises (i) an insert comprising the amino acid sequence of TLAVPFK (SEQ ID NO: 1262), optionally wherein the insert is present immediately subsequent to position 588, relative to a reference sequence numbered according to SEQ ID NO:138; and (ii) the amino acid substitutions of A587D and Q588G, numbered according to SEQ ID NO:138.

128. The AAV particle of any one of embodiments 122-127, wherein the capsid protein comprises any of the capsid proteins listed in Table 1 or a functional variant thereof.

129. The AAV particle of any one of embodiments 122-128, wherein the capsid protein comprises a VOY101, VOY201, AAVPHP.N (PHP.N), AAVPHP.B (PHP.B), AAVPHP.A (PHP.A), PHP.B2, PHP.B3, G2B4, G2B5, AAV5, AAV9, AAVrh10, or a functional variant thereof (e.g., an AAV9 capsid or variant thereof or an AAV5 capsid or a variant thereof).

130. The AAV particle of any one of embodiments 122-129, wherein:

(i) the capsid protein comprises the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto;

(ii) the capsid protein comprises an amino acid sequence comprising at least one, two, or three modifications but no more than 30, 20, or 10 modifications, e.g., substitutions, relative to the amino acid sequence of SEQ ID NO: 1;

(iii) the capsid protein comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 2 or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; and/or

(iv) the nucleotide sequence encoding the capsid protein comprises the nucleotide sequence of SEQ ID NO: 2, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto.

131. The AAV particle of any one of embodiments 122-130, wherein the capsid protein comprises:

(i) a VP1 polypeptide, VP2 polypeptide, VP3 polypeptide, or a combination thereof;

(ii) the amino acid sequence corresponding to positions 138-743, e.g., a VP2, of SEQ ID NO: 1, or a sequence with at least 80% (e.g., at least about 85, 90, 92, 95, 96, 97, 98, or 99%) sequence identity thereto;

(iii) the amino acid sequence corresponding to positions 203-743, e.g., a VP3, of SEQ ID NO: 1, or a sequence with at least 80% (e.g., at least about 85, 90, 92, 95, 96, 97, 98, or 99%) sequence identity thereto; and/or

(iv) the amino acid sequence corresponding to positions 1-743, e.g., a VP1, of SEQ ID NO: 1, or a sequence with at least 80% (e.g., at least about 85, 90, 92, 95, 96, 97, 98, or 99%) sequence identity thereto.

132. The AAV particle of any one of embodiments 122-131, wherein the nucleotide sequence encoding the capsid protein comprises:

- (i) a CTG initiation codon; and/or
- (ii) the nucleotide sequence of SEQ ID NO: 137 which comprises 3-20 mutations, e.g., substitutions, e.g., 3-15 mutations, 3-10 mutations, 3-5 mutations, 5-20 mutations, 5-15 mutations, 5-10 mutations, 10-20 mutations, 10-15 mutations, 15-20 mutations, 3 mutations, 5 mutations, 10 mutations, 12 mutations, 15 mutations, 18 mutations, or 20 mutations.

133. A vector comprising the isolated nucleic acid of any one of embodiments 1-6 or the viral genome of any one of embodiments 7-121.

134. A cell comprising the viral genome of any one of embodiments 7-11, the viral particle of any one of embodiments 122-132, or the vector of embodiment 133.

135. The cell of embodiment 134, which a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell.

136. A nucleic acid comprising the viral genome of any one of embodiments 7-121, and a backbone region suitable for replication of the viral genome in a cell, e.g., a bacterial cell (e.g., wherein the backbone region comprises one or both of a bacterial origin of replication and a selectable marker).

137. The nucleic acid of embodiment 136, wherein the viral genome comprises a nucleotide sequence of SEQ ID NO: 2006 or SEQ ID NO: 2007, or a sequence at least 97% identical thereto.

138. A method of making a viral genome, the method comprising:

- (i) providing the nucleic acid molecule comprising the viral genome embodiment 136 or 137, or a nucleic acid encoding the viral genome of any one of embodiments 7-121; and
- (ii) excising the viral genome from the backbone region, e.g., by cleaving the nucleic acid molecule upstream and downstream of the viral genome.

139. A method of making a recombinant AAV particle, the method comprising

(i) providing a host cell comprising the viral genome of any one of embodiments 7-122 or the nucleic acid encoding the viral genome of embodiment 136 or 137; and

(ii) incubating the host cell under conditions suitable to enclose the viral genome in a capsid protein, e.g., a VOY101 capsid protein, an AAV9 capsid protein or variant thereof, or an AAV5 capsid protein or variant thereof;

thereby making the isolated AAV particle.

140. The method of embodiment 139, further comprising, prior to step (i), introducing a first nucleic acid molecule comprising the viral genome into the host cell.

141. The method of embodiment 139 or 140, wherein the host cell comprises a second nucleic acid encoding a capsid protein, e.g., a VOY101 capsid protein.

142. The method of embodiment 140, further comprising introducing the second nucleic acid into the cell.

143. The method of embodiment 141 or 142, wherein the second nucleic acid molecule is introduced into the host cell prior to, concurrently with, or after the first nucleic acid molecule.

144. The method of any one of embodiments 139-143, wherein the host cell comprises a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell.

145. A pharmaceutical composition comprising the AAV particle of any one of embodiments 122-132, or an AAV particle comprising the viral genome of any one of embodiments 7-121, and a pharmaceutically acceptable excipient.

146. A method of delivering a nucleic acid sequence encoding a GBA1 protein to a subject, comprising administering an effective amount of the pharmaceutical composition of embodiment 145, the AAV particle of any one of embodiments 122-132, an AAV particle comprising the viral genome of any one of embodiments 7-121, or an AAV particle comprising a viral genome comprising the nucleic acid of any one of embodiments 1-6, thereby delivering the nucleic acid encoding a GBA1 protein to the subject.

147. The method of embodiment 146, wherein the subject has, has been diagnosed with having, or is at risk of having a disease associated with expression of GBA, e.g., aberrant or reduced GBA1 expression, e.g., expression of a GBA1 gene, GBA1 mRNA, and/or GBA1 protein.

148. The method of embodiment 146 or 147, wherein the subject has, has been diagnosed with having, or is at risk of having a neurodegenerative or neuromuscular disorder.

149. A method of treating a subject having or diagnosed with having a disease associated with GBA1 expression comprising administering an effective amount of the pharmaceutical composition of embodiment 145, the AAV particle of any one of embodiments 122-132, an AAV particle comprising the viral genome of any one of embodiments 7-121, or an AAV particle comprising a viral genome comprising the nucleic acid of any one of embodiments 1-6, thereby treating the disease associated with GBA1 expression in the subject.

150. A method of treating a subject having or diagnosed with having a neurodegenerative or neuromuscular disorder, comprising administering an effective amount of the pharmaceutical composition of embodiment 145, the AAV particle of any one of embodiments 122-132, an AAV particle comprising the viral genome of any one of embodiments 7-121, or an AAV particle comprising a viral genome comprising the nucleic acid of any one of embodiments 1-6, thereby treating the neurodegenerative or neuromuscular disorder in the subject.

151. The method of any one of embodiments 147-150, wherein the disease associated with expression of GBA1 or the neurodegenerative or neuromuscular disorder comprises Parkinson's Disease (PD), dementia with Lewy Bodies (DLB), Gaucher disease (GD), Spinal muscular atrophy (SMA), Multiple System Atrophy (MSA), or Multiple sclerosis (MS).

152. A method of treating a subject having or diagnosed with having Parkinson's Disease (PD) (e.g., PD associated with a mutation in a GBA1 gene) comprising administering an effective amount of the pharmaceutical composition of embodiment 145, the AAV particle of any one of embodiments 122-132, an AAV particle comprising the viral genome of any one of embodiments 7-121, or an AAV particle comprising a viral genome comprising the nucleic acid of any one of embodiments 1-6, thereby treating PD in the subject.

153. The method of embodiment 151 or embodiment 152, wherein the subject has one or more mutations in GBA1.

154. The method of any one of embodiments 151-153, wherein the PD is an early onset PD (e.g., before 50 years of age) or a juvenile PD (e.g., before 20 years of age).

155. The method of embodiment 151-154, wherein the PD is a tremor dominant, postural instability gait difficulty PD (PIGD) or a sporadic PD (e.g., a PD not associated with a mutation).

156. A method of treating a subject having or diagnosed with having Gaucher Disease (GD) comprising administering an effective amount of the pharmaceutical composition of embodiment 145, the AAV particle of any one of embodiments 122-132, an AAV particle comprising the viral genome of any one of embodiments 7-121, or an AAV particle comprising a viral genome comprising the nucleic acid of any one of embodiments 1-6, thereby treating GD in the subject.

157. The method of embodiment 151 or 156, wherein the GD is neuronopathic GD (e.g., affect a cell or tissue of the CNS, e.g., a cell or tissue of the brain and/or spinal cord), non-neuronopathic GD (e.g., does not affect a cell or tissue of the CNS), or combination thereof.

158. The method of any one of embodiments 151 or 156-157, wherein the GD is Type I GD (GD1), Type 2 GD (GD2), or Type 3 GD (GD3).

159. The method of embodiment 158, wherein the GD1 is non-neuronopathic GD.

160. The method of embodiment 158, wherein the GD2 is a neuronopathic GD.

161. The method of any one of embodiments 146-160, wherein the subject has a reduced level of GCase activity as compared to a reference level, when measured by an assay, e.g., an assay as described in Example 7.

162. The method of embodiment 161, wherein the reference level comprises the level of GCase activity in a subject that does not have a disease associated with GBA1 expression, a neuromuscular disorder, and/or a neurodegenerative disorder.

163. The method of any one of embodiments 149-162, wherein treating comprises prevention or progression of the disease in the subject.

164. The method of any one of embodiments 149-162, wherein treating results in amelioration of at least one symptom of the disease associated with GBA1 expression, the neurodegenerative disorder, and/or the neuromuscular disorder in the subject.

165. The method of embodiment 164, wherein the symptom of the disease associated with GBA1 expression, the neurodegenerative disorder, and/or the neuromuscular disorder comprises reduced GCase activity, accumulation of glucocerebroside and other glycolipids, e.g., within immune cells (e.g., macrophages), build-up of synuclein aggregates (e.g., Lewy bodies), developmental delay, progressive encephalopathy, progressive dementia, ataxia, myoclonus, oculomotor dysfunction, bulbar palsy, generalized weakness, trembling of a limb, depression, visual hallucinations, cognitive decline, or a combination thereof.

166. The method of any one of embodiments 146-165, wherein the subject is a human.

167. The method of any one of embodiments 146-166, wherein the subject is a juvenile, e.g., between 6 years of age to 20 years of age.

168. The method of any one of embodiments 146-167, wherein the subject is an adult, e.g., above 20 years of age.

169. The method of any one of embodiments 146-168, wherein the subject has a mutation in a GBA1 gene, GBA1 mRNA, and/or GBA1 protein.

170. The method of any one of embodiments 146-169, wherein the AAV particle is administered to the subject intravenously, intracerebrally, via intrathalamic (ITH) administration, intramuscularly, intrathecally, intracerebroventricularly, via intraparenchymal administration, via focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles

(FUS-MB), or MRI-guided FUS coupled with intravenous administration, or via intra-cisterna magna injection (ICM).

171. The method of any one of embodiments 146-170, wherein the AAV particle is administered via dual ITH and ICM administration.

172. The method of any one of embodiments 146-170, wherein the AAV particle is administered via intravenous injection, optionally wherein the intravenous injection is via focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration.

173. The method of any one of embodiments 146-172, wherein the AAV particle is administered to a cell, tissue, or region of the CNS, e.g., a region of the brain or spinal cord, e.g., the parenchyma, the cortex, substantia nigra, caudate cerebellum, striatum, corpus callosum, cerebellum, brain stem caudate-putamen, thalamus, superior colliculus, the spinal cord, or a combination thereof.

174. The method of any one of embodiments 146-173, wherein the AAV particle is administered to a cell, tissue, or region of the periphery, e.g., a lung cell or tissue, a heart cell or tissue, a spleen cell or tissue, a liver cell or tissue, or a combination thereof.

175. The method of any one of embodiments 146-174, wherein the AAV particle is administered to the cerebral spinal fluid, the serum, or a combination thereof.

176. The method of any one of embodiments 146-175, wherein the AAV particle is administered to at least two tissues, or regions of the CNS, e.g., bilateral administration.

177. The method of any one of embodiments 146-176, further comprising performing a blood test, performing an imaging test, collecting a CNS biopsy sample, collecting a tissue biopsy, (e.g., a biopsy of the lung, liver, or spleen), collecting a blood or serum sample, or collecting an aqueous cerebral spinal fluid biopsy.

178. The method of any one of embodiments 146-177, which further comprises evaluating, e.g., measuring, the level of GBA1 expression, e.g., GBA1 gene, GBA1 mRNA, and/or GBA1

protein expression, in the subject, e.g., in a cell, tissue, or fluid, of the subject, optionally wherein the level of GBA1 protein is measured by an assay described herein, e.g., an ELISA, a Western blot, or an immunohistochemistry assay.

179. The method of embodiment 178, wherein measuring the level of GBA1 expression is performed prior to, during, or subsequent to treatment with the AAV particle.

180. The method of embodiment 178 or 179, wherein the cell or tissue is a cell or tissue of the central nervous system (e.g., parenchyma) or a peripheral cell or tissue (e.g., the liver, heart, and/or spleen).

181. The method of any one of embodiments 146-180, wherein the administration results in increased level of GBA1 protein expression in a cell or tissue of the subject, relative to reference level, e.g., a subject that has not received treatment, e.g., has not been administered the AAV particle.

182. The method of any one of embodiments 146-181, which further comprises evaluating, e.g., measuring, the level of GCase activity in the subject, e.g., in a cell or tissue of the subject, optionally wherein the level of GCase activity is measured by an assay described herein, e.g., assay as described in Example 7.

183. The method of any one of embodiments 146-182, wherein the administration results in an increase in at least one, two, or all of:

(i) the level of GCase activity in a cell, tissue, (e.g., a cell or tissue of the CNS, e.g., the cortex, striatum, thalamus, cerebellum, and/or brainstem), and/or fluid (e.g., CSF and/or serum), of the subject, optionally wherein the level of GCase activity is increased by at least 2, 3, 4, or 5 fold, as compared to a reference level, e.g., a subject that has not received treatment, e.g., has not been administered the AAV particle;

(ii) the level of viral genomes (VG) per cell in a CNS tissue (e.g., the cortex, striatum, thalamus, cerebellum, brainstem, and/or spinal cord) of the subject, optionally wherein the VG level is increased by greater than 50 VGs per cell, as compared to a peripheral tissue, wherein the level of VGs per cell is at least 4-10 fold lower than the levels in the CNS tissue, e.g., as measured by an assay as described herein; and/or

(iii) the level of GBA1 mRNA expression in a cell or tissue (e.g. a cell or tissue of the CNS, e.g., the cortex, thalamus, and/or brainstem), optionally wherein the level of GBA1 mRNA is increased by at least 100-1300 fold, e.g., 100 fold, 200 fold, 500 fold, 600 fold, 850 fold, 900 fold, 950 fold, 1000 fold, 1050 fold, 1100 fold, 1150 fold, 1200 fold, 1250 fold, or 1300 fold as compared to a reference level, e.g., a subject that has not received treatment (e.g., has not been administered the AAV particle), or endogenous GBA1 mRNA levels, e.g., as measured by an assay as described herein.

184. The method of any one of embodiments 146-183, wherein further comprising administration of an additional therapeutic agent and/or therapy suitable for treatment or prevention of the disease associated GBA1 expression, the neurodegenerative disorder, and/or the neuromuscular disorder.

185. The method of embodiment 184, wherein the additional therapeutic agent comprises enzyme replacement therapy (ERT) (e.g., imiglucerase, velaglucerase alfa, or taliglucerase alfa); substrate reduction therapy (SRT) (e.g., eliglustat or miglustat), blood transfusion, levodopa, carbidopa, Safinamide, dopamine agonists (e.g., pramipexole, rotigotine, or ropinirole), anticholinergics (e.g., benztropine or trihexyphenidyl), cholinesterase inhibitors (e.g., rivastigmine, donepezil, or galantamine), an N-methyl-d-aspartate (NMDA) receptor antagonist (e.g., memantine), or a combination thereof.

186. The isolated nucleic acid of any one of embodiments 1-6, the viral genome of any one of embodiments 7-121, the AAV particle of any one of embodiments 122-132, or the pharmaceutical composition of embodiment 145 for use in the manufacture of a medicament.

187. The isolated nucleic acid of any one of embodiments 1-6, the viral genome of any one of embodiments 7-121, the AAV particle of any one of embodiments 122-132, or the pharmaceutical composition of embodiment 145 or use in the treatment of a disease associated with GBA1 expression, a neuromuscular and/or a neurodegenerative disorder.

188. Use of an effective amount of an AAV particle comprising the genome of any one of embodiments 7-121, an AAV particle comprising a genome comprising the nucleic acid of any one of embodiments 1-6, the AAV particle of any one of embodiments 122-132, or the pharmaceutical composition of embodiment 146, in the manufacture of a medicament for the

treatment of a disease associated with GBA1 expression, a neuromuscular and/or a neurodegenerative disorder.

189. An adeno-associated virus (AAV) viral genome comprising the nucleotide sequence of SEQ ID NO: 2006 or 2007.

190. An AAV particle comprising the AAV viral genome of claim 189 and a capsid selected from a group consisting of those listed in Table 1.

191. The viral genome of embodiment 190, wherein the capsid comprises an AAV2 serotype, AAV5 serotype, or AAV9 serotype, or a variant thereof.

192. A pharmaceutical composition comprising the AAV particle of claim 190 or claim 191.

193. A method of treating a neurological or neuromuscular disorder, said method comprising administering to a subject the pharmaceutical composition of claim 192.

194. The method of claim 193, wherein the neurological or neuromuscular disorder is Parkinson's Disease, Gaucher disease, or Dementia with Lewy Bodies, or a related disorder.

195. The method of claim 194, wherein the neurological or neuromuscular disorder is a disorder associated with decreased GCase protein levels.

[033] The details of various aspects or embodiments of the present disclosure are set forth below. Other features, objects, and advantages of the disclosure will be apparent from the description and the claims. In the description, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art in the field of this disclosure. In the case of conflict, the present description will control.

BRIEF DESCRIPTION OF THE DRAWINGS

[034] **FIGs. 1A-1B** depict LC-MS/MS results quantifying levels of GBA1 substrate glucosylsphingosine (GlcSph) in cell lysates of Gaucher disease patient derived fibroblasts (GD1 patient GM04394, GD1 Patient GM00852, and GD2 patient GM00877) and healthy control fibroblasts (CLT GM05758, CTL GM02937 and CTL GM08402). Data are shown as

GlcSph normalized to actin (**FIG. 1A**) or normalized to lysosomal protein Lamp1 (**FIG 1B**). **FIG. 1C** depicts GBA1 protein levels detected in lysates of Gaucher patient-derived fibroblasts (GD1 and GD2) compared to healthy control fibroblast (HC) by LC-MS/MS. Data are shown as concentration of GBA1 protein (ng) relative to total protein (mg).

[035] **FIGs. 2A-2B** depict GCase activity (RFU/mL normalized to mg of protein) in GD-II GM00877 fibroblast cell pellets (**FIG. 2A**) or conditioned media (**FIG. 2B**) at Day 7 after transduction with AAV2 viral particles comprising the viral genome construct on the X-axis from left to right: GBA_VG1 (SEQ ID NO: 1759), GBA_VG9 (SEQ ID NO: 1767), GBA_VG10 (SEQ ID NO: 1768), GBA_VG11 (SEQ ID NO: 1769), GBA_VG6 (SEQ ID NO: 1764), GBA_VG7 (SEQ ID NO: 1765), GBA_VG12 (SEQ ID NO: 1770), GBA_VG3 (SEQ ID NO: 1761), GBA_VG4 (SEQ ID NO: 1762), GBA_VG5 (SEQ ID NO: 1763), and GBA_VG13 (SEQ ID NO: 1771), at MOI of $10^{3.5}$. The dotted line indicates the baseline level (vehicle treatment).

[036] **FIG. 3** depicts levels of GBA1 substrate glucosylsphingosine (GlcSph) in the cell lysates (ng/mg Lamp1) collected from GD-II patient fibroblasts (GM00877) at Day 7 after transduction with transduction of a no AAV control or AAV2 vectors comprising the viral genome indicated on the X-axis (from left to right: GBA_VG1 (SEQ ID NO: 1759), GBA_VG9 (SEQ ID NO: 1767), GBA_VG6 (SEQ ID NO: 1764), GBA_VG7 (SEQ ID NO: 1765), GBA_VG3 (SEQ ID NO: 1761), GBA_VG4 (SEQ ID NO: , and GBA_VG5(SEQ ID NO: 1763)).

[037] **FIG. 4A** depicts GCase activity measured as RFU per mL normalized to mg of protein in GD-II patient fibroblasts (GD-II GM00877) on day 7 post-transduction with AAV2 vectors comprising the viral genome indicated on the X-axis (from left to right: GBA_VG1 (SEQ ID NO: 1759), GBA_VG14 (SEQ ID NO: 1809), GBA_VG15 (SEQ ID NO: 1810), GBA_VG16 (SEQ ID NO: 1811), GBA_VG17 (SEQ ID NO: 1812), GBA_VG18 (SEQ ID NO: 1813), GBA_VG19 (SEQ ID NO: 1814), and GBA_VG20 (SEQ ID NO: 1815)) at an MOI of $10^{2.5}$ (first bar), 10^3 (second bar), $10^{3.5}$ and 10^4 (third bar). **FIG. 4B** depicts the level of the GBA1 substrate glucosylsphingosine (GlcSph, ng/mg Lamp1) in the cell lysate from GD-II patient-derived fibroblasts at day 7 after transduction with AAV2 vectors comprising the viral genome indicated on the X-axis (from left to right: GBA_VG1 (SEQ ID NO: 1759), GBA_VG14 (SEQ ID NO: 1809), GBA_VG15 (SEQ ID NO: 1810), GBA_VG16 (SEQ ID NO: 1811), GBA_VG17 (SEQ ID NO: 1812), GBA_VG18 (SEQ ID NO: 1813), GBA_VG19 (SEQ ID NO: 1814), and GBA_VG20 (SEQ ID NO: 1815)) at an MOI of $10^{2.5}$ (first bar), 10^3 (second bar), $10^{3.5}$ and 10^4 (third bar).

[038] **FIG. 5** depicts the GC content and distribution of a first codon-optimized nucleotide sequence encoding a GBA1 protein of SEQ ID NO: 1773, a second codon-optimized nucleotide sequence encoding a GBA1 protein of SEQ ID NO: 1781, and a wild-type nucleotide sequence encoding a GBA1 protein of SEQ ID NO: 1777.

[039] **FIGs. 6A-6B** compare activity of a GBA1 protein expressed by AAV2 vectorized viral genome constructs: GBA_VG1 (SEQ ID NO: 1759), GBA_VG17 (SEQ ID NO: 1812), and GBA_VG21 (SEQ ID NO: 1816). **FIG. 6A** depicts the GCase activity (RFU/mL) normalized to mg of protein in GD-II patient fibroblasts treated with AAV2 viral particles at an MOI of $10^{4.5}$, comprising the viral genome constructs indicated on the X-axis (GBA_VG1 (SEQ ID NO: 1759), GBA_VG17 (SEQ ID NO: 1812), and GBA_VG21 (SEQ ID NO: 1816)) compared to a no AAV control. **FIG. 6B** depicts glucosylsphingosine (GlcSph) (ng/mL Lamp1) in the cell lysate from GD-II patient fibroblasts treated with AAV2 viral particles comprising the viral genome constructs indicated on the X-axis (from left to right GBA_VG1 (SEQ ID NO: 1759), GBA_VG17 (SEQ ID NO: 1812), and GBA_VG21 (SEQ ID NO: 1816)) at an MOI of 10^6 , or a no AAV treatment control.

[040] **FIG. 7** depicts the GCase activity (RFU/mL) per mg of protein in rat embryonic dorsal root ganglion (DRG) neurons transduced an AAV2 vector comprising GBA_VG33 (SEQ ID NO: 1828) or an AAV2 vector comprising GBA_VG17 (SEQ ID NO: 1812) at an MOI of $10^{3.5}$ or $10^{4.5}$, compared to a no AAV control.

[041] **FIG. 8** depicts the biodistribution (VG/cell) versus GCase activity (RFU/mL, fold over endogenous GCase activity, normalized to mg of protein) in the cortex, striatum, thalamus, brainstem, cerebellum, and liver in wild-type mice at one-month post-IV injection of VOY101.GBA_VG17 (SEQ ID NO: 1812) at 2×10^{13} vg/kg.

[042] **FIG. 9** depicts the biodistribution (VG/cell) in the cortex, striatum, and brainstem of wild-type mice at 28 days post-IV injection of VOY101.GBA_VG17 (SEQ ID NO: 1812), VOY101.GBA_VG35 (SEQ ID NO: 2006) or VOY101.GBA_VG36 (SEQ ID NO: 2007).

[043] **FIG. 10** depicts the GCase activity in the cortex, striatum, and brainstem of wild-type mice at 28 days post-IV injection of VOY101.GBA_VG17 (SEQ ID NO: 1812), VOY101.GBA_VG35 (SEQ ID NO: 2006) or VOY101.GBA_VG36 (SEQ ID NO: 2007).

[044] **FIG. 11** depicts the biodistribution, mRNA expression, and GCase activities in the brainstem and DRGs of wild-type mice at 28 days post-IV injection of VOY101.GBA_VG17 (SEQ ID NO: 1812), VOY101.GBA_VG35 (SEQ ID NO: 2006) or VOY101.GBA_VG36 (SEQ ID NO: 2007).

[045] **FIG. 12** depicts the substrate quantification of glucosylceramide and glucosylsphingosine by LC-MS/MS in the brainstem, striatum, and DRGs of wild-type mice at 28 days post-IV injection of VOY101.GBA_VG17 (SEQ ID NO: 1812), VOY101.GBA_VG35 (SEQ ID NO: 2006) or VOY101.GBA_VG36 (SEQ ID NO: 2007).

[046] **FIG. 13** depicts biodistribution (VG/cell) in the cortex and GCase activities in the cortex, striatum, and brainstem of wild-type mice at 28 days post-IV injection of VOY101.GBA_VG17 (SEQ ID NO: 1812) or VOY101.GBA_VG17-HA.

[047] **FIG. 14A** depicts immunohistochemical analysis of HA expression in the cortex, striatum, and brainstem of wild-type mice at 28 days post-IV injection of VOY101.GBA_VG17 (SEQ ID NO: 1812) or VOY101.GBA_VG17-HA. **FIG. 14B** depicts immunohistochemical analysis of HA expression in the cerebellum, thalamus, and hippocampus of wild-type mice at 28 days post-IV injection of VOY101.GBA_VG17 (SEQ ID NO: 1812) or VOY101.GBA_VG17-HA.

DETAILED DESCRIPTION

Overview

[048] Described herein, inter alia, are compositions comprising isolated, e.g., recombinant, viral particles, e.g., AAV particles, for delivery, e.g., vectorized delivery, of a protein, e.g., a GBA1 protein, and methods of making and using the same. Adeno-associated viruses (AAV) are small non-enveloped icosahedral capsid viruses of the Parvoviridae family characterized by a single stranded DNA viral genome. Parvoviridae family viruses consist of two subfamilies: Parvovirinae, which infect vertebrates, and Densovirinae, which infect invertebrates. The Parvoviridae family includes the Dependovirus genus which includes AAV, capable of replication in vertebrate hosts including, but not limited to, human, primate, bovine, canine, equine, and ovine species.

[049] The parvoviruses and other members of the Parvoviridae family are generally described in Kenneth I. Berns, "Parvoviridae: The Viruses and Their Replication," Chapter 69 in Fields Virology (3d Ed. 1996), the contents of which are incorporated by reference in their entirety.

[050] AAV have proven to be useful as a biological tool due to their relatively simple structure, their ability to infect a wide range of cells (including quiescent and dividing cells) without integration into the host genome and without replicating, and their relatively benign immunogenic profile. The genome of the virus may be manipulated to contain a minimum of components for the assembly of a functional recombinant virus, or viral particle, which is loaded

with or engineered to target a particular tissue and express or deliver a desired payload. The genome of the virus may be modified to contain a minimum of components for the assembly of a functional recombinant virus, or viral particle, which is loaded with or engineered to express or deliver a desired nucleic acid construct or payload, *e.g.*, a transgene, polypeptide-encoding polynucleotide, *e.g.*, a GBA1 protein, *e.g.*, a GCase, GCase and PSAP, GCase and SapA, or GCase and SapC, GCase and a cell penetration peptide (*e.g.*, an ApoEII peptide, a TAT peptide, or an ApoB peptide), or GCase and a lysosomal targeting sequence (LTS), which may be delivered to a target cell, tissue, or organism. In some embodiments, the genome encodes a wildtype GBA1 protein. In some embodiments, the genome comprises a codon-optimized, CpG-reduced (*e.g.*, CpG-depleted) nucleotide sequence encoding a wildtype GBA1 protein, *e.g.*, as compared to a wildtype GBA1 encoding sequence (*e.g.*, comprising the nucleotide sequence of SEQ ID NO: 1776 or 1777). In some embodiments, the target cell is a CNS cell. In some embodiments, the target tissue is a CNS tissue. The target CNS tissue may be brain tissue. In some embodiments, the brain target comprises caudate, putamen, thalamus, superior colliculus, cortex, and corpus collosum.

[051] Gene therapy presents an alternative approach for Parkinson's Disease (PD) and related diseases sharing single-gene etiology, such as Gaucher disease and Dementia with Lewy Bodies and related disorders. AAVs are commonly used in gene therapy approaches as a result of a number of advantageous features. Without wishing to be bound by theory, it is believed in some embodiments, that expression vectors, *e.g.*, an adeno-associated viral vector (AAVs) or AAV particle, *e.g.*, an AAV particle described herein, can be used to administer and/or deliver a GBA1 protein (*e.g.*, GCase and related proteins), in order to achieve sustained, high concentrations, allowing for longer lasting efficacy, fewer dose treatments, broad biodistribution, and/or more consistent levels of the GBA1 protein, relative to a non-AAV therapy.

[052] As demonstrated in the Examples herein below, the compositions and methods described herein provides improved features compared to prior enzyme replacement approaches, including (i) increased GCase activity in a cell, tissue, (*e.g.*, a cell or tissue of the CNS, *e.g.*, the cortex, striatum, thalamus, cerebellum, and/or brainstem), and/or fluid (*e.g.*, CSF and/or serum), of the subject; (ii) increased biodistribution throughout the CNS (*e.g.*, the cortex, striatum, thalamus, cerebellum, brainstem, and/or spinal cord), and the periphery (*e.g.*, the liver), and/or (iii) elevated payload expression, *e.g.*, GBA1 mRNA expression, in multiple brain regions (*e.g.*, cortex, thalamus, and brain stem) and the periphery (*e.g.*, the liver). In some embodiments, an AAV viral genome comprising a codon-optimized, CpG-reduced (*e.g.*, CpG-depleted)

nucleotide sequence encoding a GBA1 protein (e.g., SEQ ID NO: 2001 or SEQ ID NO: 2002) results in high biodistribution in the CNS; increased GCase activity in the CNS, peripheral tissues, and/or fluid; and successful transgene transcription and expression. The compositions and methods described herein can be used in the treatment of disorders associated with a lack of a GBA1 protein and/or GCase activity, such as neuronopathic (affects the CNS) and non-neuronopathic (affects non-CNS) Gaucher's disease (e.g., Type 1 GD, Type 2 GD, or Type 3 GD), a PD associated with a mutation in a GBA1 gene, and a dementia with Lewy Bodies (DLB). In some embodiments, the disclosure provides an AAV viral genome comprising a codon-optimized, CpG-reduced (e.g., CpG-depleted) nucleotide sequence encoding a GBA1 protein (e.g., comprising the nucleotide sequence of SEQ ID NO: 2001 or SEQ ID NO: 2002) that has reduced immunogenicity compared to a codon-optimized sequence comprising one or more or all CpG motifs.

I. Compositions

Adeno-associated viral (AAV) vectors

[053] AAV have a genome of about 5,000 nucleotides in length which contains two open reading frames encoding the proteins responsible for replication (Rep) and the structural protein of the capsid (Cap). The open reading frames are flanked by two Inverted Terminal Repeat (ITR) sequences, which serve as the origin of replication of the viral genome. The wild-type AAV viral genome comprises nucleotide sequences for two open reading frames, one for the four non-structural Rep proteins (Rep78, Rep68, Rep52, Rep40, encoded by Rep genes) and one for the three capsid, or structural, proteins (VP1, VP2, VP3, encoded by capsid genes or Cap genes). The Rep proteins are important for replication and packaging, while the capsid proteins are assembled to create the protein shell of the AAV, or AAV capsid. Alternative splicing and alternate initiation codons and promoters result in the generation of four different Rep proteins from a single open reading frame and the generation of three capsid proteins from a single open reading frame. Though it varies by AAV serotype, as a non-limiting example, for AAV9/hu.14 (SEQ ID NO: 123 of US 7,906,111, the contents of which are herein incorporated by reference in their entirety) VP1 refers to amino acids 1-736, VP2 refers to amino acids 138-736, and VP3 refers to amino acids 203-736. As another non-limiting example, VP1 refers to amino acids 1-743 numbered according to SEQ ID NO: 1, VP2 refers to amino acids 138-743 numbered according to SEQ ID NO: 1, and VP3 refers to amino acids 203-743 numbered according to SEQ ID NO: 1. In other words, VP1 is the full-length capsid sequence, while VP2 and VP3 are shorter components of the whole. As a result, changes in the sequence in the VP3 region, are also changes to VP1 and VP2, however, the percent difference as compared to the parent

sequence will be greatest for VP3 since it is the shortest sequence of the three. Though described here in relation to the amino acid sequence, the nucleic acid sequence encoding these proteins can be similarly described. Together, the three capsid proteins assemble to create the AAV capsid protein. While not wishing to be bound by theory, the AAV capsid protein typically comprises a molar ratio of 1:1:10 of VP1:VP2:VP3. As used herein, an “AAV serotype” is defined primarily by the AAV capsid. In some instances, the ITRs are also specifically described by the AAV serotype (*e.g.*, AAV2/9).

[054] The AAV vector typically requires a co-helper (*e.g.*, adenovirus) to undergo productive infection in cells. In the absence of such helper functions, the AAV virions essentially enter host cells but do not integrate into the cells' genome.

[055] AAV vectors have been investigated for delivery of gene therapeutics because of several unique features. Non-limiting examples of the features include (i) the ability to infect both dividing and non-dividing cells; (ii) a broad host range for infectivity, including human cells; (iii) wild-type AAV has not been associated with any disease and has not been shown to replicate in infected cells; (iv) the lack of cell-mediated immune response against the vector, and (v) the non-integrative nature in a host chromosome thereby reducing potential for long-term genetic alterations. Moreover, infection with AAV vectors has minimal influence on changing the pattern of cellular gene expression (Stilwell and Samulski *et al.*, *Biotechniques*, 2003, 34, 148, the contents of which are herein incorporated by reference in their entirety).

[056] Typically, AAV vectors for GCaCase protein delivery may be recombinant viral vectors which are replication defective as they lack sequences encoding functional Rep and Cap proteins within the viral genome. In some cases, the defective AAV vectors may lack most or all coding sequences and essentially only contain one or two AAV ITR sequences and a payload sequence. In certain embodiments, the viral genome encodes GCaCase protein. In some embodiments, the viral genome encodes GCaCase protein and SapA protein. In some embodiments, the viral genome encodes GCaCase protein and SapC protein. For example, the viral genome can encode human GCaCase, human GCaCase+SapA, or human GCaCase+SapC protein(s).

[057] In some embodiments, the viral genome may comprise one or more lysosomal targeting sequences (LTS).

[058] In some embodiments, the viral genome may comprise one or more cell penetrating peptide sequences (CPP).

[059] In some embodiments, a viral genome may comprise one or more lysosomal targeting sequences and one or more cell penetrating sequences.

[060] In some embodiments, the AAV particles of the present disclosure may be introduced

into mammalian cells.

[061] AAV vectors may be modified to enhance the efficiency of delivery. Such modified AAV vectors of the present disclosure can be packaged efficiently and can be used to successfully infect the target cells at high frequency and with minimal toxicity.

[062] In other embodiments, AAV particles of the present disclosure may be used to deliver GCa6 protein to the central nervous system (see, *e.g.*, U.S. Pat. No. 6,180,613; the contents of which are herein incorporated by reference in their entirety) or to specific tissues of the CNS.

[063] As used herein, the term “AAV vector” or “AAV particle” comprises a capsid and a viral genome comprising a payload. As used herein, “payload” or “payload region” refers to one or more polynucleotides or polynucleotide regions encoded by or within a viral genome or an expression product of such polynucleotide or polynucleotide region, *e.g.*, a transgene, a polynucleotide encoding a polypeptide or multi-polypeptide, *e.g.*, GCa6 protein.

[064] It is understood that the compositions described herein may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on their functions.

AAV Serotypes

[065] AAV particles of the present disclosure may comprise or be derived from any natural or recombinant AAV serotype. According to the present disclosure, the AAV particles may utilize or be based on a serotype or include a peptide selected from any of the following VOY101, VOY201, AAVPHP.B (PHP.B), AAVPHP.A (PHP.A), AAVG2B-26, AAVG2B-13, AAVTH1.1-32, AAVTH1.1-35, AAVPHP.B2 (PHP.B2), AAVPHP.B3 (PHP.B3), AAVPHP.N/PHP.B-DGT, AAVPHP.B-EST, AAVPHP.B-GGT, AAVPHP.B-ATP, AAVPHP.B-ATT-T, AAVPHP.B-DGT-T, AAVPHP.B-GGT-T, AAVPHP.B-SGS, AAVPHP.B-AQP, AAVPHP.B-QQP, AAVPHP.B-SNP(3), AAVPHP.B-SNP, AAVPHP.B-QGT, AAVPHP.B-NQT, AAVPHP.B-EGS, AAVPHP.B-SGN, AAVPHP.B-EGT, AAVPHP.B-DST, AAVPHP.B-DST, AAVPHP.B-STP, AAVPHP.B-PQP, AAVPHP.B-SQP, AAVPHP.B-QLP, AAVPHP.B-TMP, AAVPHP.B-TTP, AAVPHP.S/G2A12, AAVG2A15/G2A3 (G2A3), AAVG2B4 (G2B4), AAVG2B5 (G2B5), PHP.S, AAV1, AAV2, AAV2G9, AAV3, AAV3a, AAV3b, AAV3-3, AAV4, AAV4-4, AAV5, AAV6, AAV6.1, AAV6.2, AAV6.1.2, AAV7, AAV7.2, AAV8, AAV9, AAV9.11, AAV9.13, AAV9.16, AAV9.24, AAV9.45, AAV9.47, AAV9.61, AAV9.68, AAV9.84, AAV9.9, AAV10, AAV11, AAV12, AAV16.3, AAV24.1, AAV27.3, AAV42.12, AAV42-1b, AAV42-2, AAV42-3a, AAV42-3b, AAV42-4, AAV42-5a, AAV42-5b, AAV42-6b, AAV42-8, AAV42-10, AAV42-11, AAV42-12, AAV42-13, AAV42-15, AAV42-aa, AAV43-1, AAV43-12, AAV43-20, AAV43-21, AAV43-23, AAV43-25,

AAV43-5, AAV44.1, AAV44.2, AAV44.5, AAV223.1, AAV223.2, AAV223.4, AAV223.5, AAV223.6, AAV223.7, AAV1-7/rh.48, AAV1-8/rh.49, AAV2-15/rh.62, AAV2-3/rh.61, AAV2-4/rh.50, AAV2-5/rh.51, AAV3.1/hu.6, AAV3.1/hu.9, AAV3-9/rh.52, AAV3-11/rh.53, AAV4-8/rh.64, AAV4-9/rh.54, AAV4-19/rh.55, AAV5-3/rh.57, AAV5-22/rh.58, AAV7.3/hu.7, AAV16.8/hu.10, AAV16.12/hu.11, AAV29.3/bb.1, AAV29.5/bb.2, AAV106.1/hu.37, AAV114.3/hu.40, AAV127.2/hu.41, AAV127.5/hu.42, AAV128.3/hu.44, AAV130.4/hu.48, AAV145.1/hu.53, AAV145.5/hu.54, AAV145.6/hu.55, AAV161.10/hu.60, AAV161.6/hu.61, AAV33.12/hu.17, AAV33.4/hu.15, AAV33.8/hu.16, AAV52/hu.19, AAV52.1/hu.20, AAV58.2/hu.25, AAVA3.3, AAVA3.4, AAVA3.5, AAVA3.7, AAVC1, AAVC2, AAVC5, AAV-DJ, AAV-DJ8, AAVF3, AAVF5, AAVH2, AAVrh.72, AAVhu.8, AAVrh.68, AAVrh.70, AAVpi.1, AAVpi.3, AAVpi.2, AAVrh.60, AAVrh.44, AAVrh.65, AAVrh.55, AAVrh.47, AAVrh.69, AAVrh.45, AAVrh.59, AAVhu.12, AAVH6, AAVLK03, AAVH-1/hu.1, AAVH-5/hu.3, AAVLG-10/rh.40, AAVLG-4/rh.38, AAVLG-9/hu.39, AAVN721-8/rh.43, AAVCh.5, AAVCh.5R1, AAVcy.2, AAVcy.3, AAVcy.4, AAVcy.5, AAVCy.5R1, AAVCy.5R2, AAVCy.5R3, AAVCy.5R4, AAVcy.6, AAVhu.1, AAVhu.2, AAVhu.3, AAVhu.4, AAVhu.5, AAVhu.6, AAVhu.7, AAVhu.9, AAVhu.10, AAVhu.11, AAVhu.13, AAVhu.15, AAVhu.16, AAVhu.17, AAVhu.18, AAVhu.20, AAVhu.21, AAVhu.22, AAVhu.23.2, AAVhu.24, AAVhu.25, AAVhu.27, AAVhu.28, AAVhu.29, AAVhu.29R, AAVhu.31, AAVhu.32, AAVhu.34, AAVhu.35, AAVhu.37, AAVhu.39, AAVhu.40, AAVhu.41, AAVhu.42, AAVhu.43, AAVhu.44, AAVhu.44R1, AAVhu.44R2, AAVhu.44R3, AAVhu.45, AAVhu.46, AAVhu.47, AAVhu.48, AAVhu.48R1, AAVhu.48R2, AAVhu.48R3, AAVhu.49, AAVhu.51, AAVhu.52, AAVhu.54, AAVhu.55, AAVhu.56, AAVhu.57, AAVhu.58, AAVhu.60, AAVhu.61, AAVhu.63, AAVhu.64, AAVhu.66, AAVhu.67, AAVhu.14/9, AAVhu.t 19, AAVrh.2, AAVrh.2R, AAVrh.8, AAVrh.8R, AAVrh.10, AAVrh.12, AAVrh.13, AAVrh.13R, AAVrh.14, AAVrh.17, AAVrh.18, AAVrh.19, AAVrh.20, AAVrh.21, AAVrh.22, AAVrh.23, AAVrh.24, AAVrh.25, AAVrh.31, AAVrh.32, AAVrh.33, AAVrh.34, AAVrh.35, AAVrh.36, AAVrh.37, AAVrh.37R2, AAVrh.38, AAVrh.39, AAVrh.40, AAVrh.46, AAVrh.48, AAVrh.48.1, AAVrh.48.1.2, AAVrh.48.2, AAVrh.49, AAVrh.51, AAVrh.52, AAVrh.53, AAVrh.54, AAVrh.56, AAVrh.57, AAVrh.58, AAVrh.61, AAVrh.64, AAVrh.64R1, AAVrh.64R2, AAVrh.67, AAVrh.73, AAVrh.74, AAVrh8R, AAVrh8R A586R mutant, AAVrh8R R533A mutant, AAV, BAAV, caprine AAV, bovine AAV, AAVhE1.1, AAVhEr1.5, AAVhEr1.14, AAVhEr1.8, AAVhEr1.16, AAVhEr1.18, AAVhEr1.35, AAVhEr1.7, AAVhEr1.36, AAVhEr2.29, AAVhEr2.4, AAVhEr2.16, AAVhEr2.30, AAVhEr2.31, AAVhEr2.36, AAVhEr1.23, AAVhEr3.1, AAV2.5T, AAV-PAEC, AAV-LK01,

AAV-LK02, AAV-LK03, AAV-LK04, AAV-LK05, AAV-LK06, AAV-LK07, AAV-LK08, AAV-LK09, AAV-LK10, AAV-LK11, AAV-LK12, AAV-LK13, AAV-LK14, AAV-LK15, AAV-LK16, AAV-LK17, AAV-LK18, AAV-LK19, AAV-PAEC2, AAV-PAEC4, AAV-PAEC6, AAV-PAEC7, AAV-PAEC8, AAV-PAEC11, AAV-PAEC12, AAV-2-pre-miRNA-101, AAV-8h, AAV-8b, AAV-h, AAV-b, AAV SM 10-2, AAV Shuffle 100-1, AAV Shuffle 100-3, AAV Shuffle 100-7, AAV Shuffle 10-2, AAV Shuffle 10-6, AAV Shuffle 10-8, AAV Shuffle 100-2, AAV SM 10-1, AAV SM 10-8, AAV SM 100-3, AAV SM 100-10, BNP61 AAV, BNP62 AAV, BNP63 AAV, AAVrh.50, AAVrh.43, AAVrh.62, AAVrh.48, AAVhu.19, AAVhu.11, AAVhu.53, AAV4-8/rh.64, AAVLG-9/hu.39, AAV54.5/hu.23, AAV54.2/hu.22, AAV54.7/hu.24, AAV54.1/hu.21, AAV54.4R/hu.27, AAV46.2/hu.28, AAV46.6/hu.29, AAV128.1/hu.43, true type AAV (ttAAV), UPENN AAV 10, Japanese AAV 10 serotypes, AAV CBr-7.1, AAV CBr-7.10, AAV CBr-7.2, AAV CBr-7.3, AAV CBr-7.4, AAV CBr-7.5, AAV CBr-7.7, AAV CBr-7.8, AAV CBr-B7.3, AAV CBr-B7.4, AAV CBr-E1, AAV CBr-E2, AAV CBr-E3, AAV CBr-E4, AAV CBr-E5, AAV CBr-e5, AAV CBr-E6, AAV CBr-E7, AAV CBr-E8, AAV CHt-1, AAV CHt-2, AAV CHt-3, AAV CHt-6.1, AAV CHt-6.10, AAV CHt-6.5, AAV CHt-6.6, AAV CHt-6.7, AAV CHt-6.8, AAV CHt-P1, AAV CHt-P2, AAV CHt-P5, AAV CHt-P6, AAV CHt-P8, AAV CHt-P9, AAV CKd-1, AAV CKd-10, AAV CKd-2, AAV CKd-3, AAV CKd-4, AAV CKd-6, AAV CKd-7, AAV CKd-8, AAV CKd-B1, AAV CKd-B2, AAV CKd-B3, AAV CKd-B4, AAV CKd-B5, AAV CKd-B6, AAV CKd-B7, AAV CKd-B8, AAV CKd-H1, AAV CKd-H2, AAV CKd-H3, AAV CKd-H4, AAV CKd-H5, AAV CKd-H6, AAV CKd-N3, AAV CKd-N4, AAV CKd-N9, AAV CLg-F1, AAV CLg-F2, AAV CLg-F3, AAV CLg-F4, AAV CLg-F5, AAV CLg-F6, AAV CLg-F7, AAV CLg-F8, AAV CLv-1, AAV CLv1-1, AAV CLv1-10, AAV CLv1-2, AAV CLv-12, AAV CLv1-3, AAV CLv-13, AAV CLv1-4, AAV CLv1-7, AAV CLv1-8, AAV CLv1-9, AAV CLv-2, AAV CLv-3, AAV CLv-4, AAV CLv-6, AAV CLv-8, AAV CLv-D1, AAV CLv-D2, AAV CLv-D3, AAV CLv-D4, AAV CLv-D5, AAV CLv-D6, AAV CLv-D7, AAV CLv-D8, AAV CLv-E1, AAV CLv-K1, AAV CLv-K3, AAV CLv-K6, AAV CLv-L4, AAV CLv-L5, AAV CLv-L6, AAV CLv-M1, AAV CLv-M11, AAV CLv-M2, AAV CLv-M5, AAV CLv-M6, AAV CLv-M7, AAV CLv-M8, AAV CLv-M9, AAV CLv-R1, AAV CLv-R2, AAV CLv-R3, AAV CLv-R4, AAV CLv-R5, AAV CLv-R6, AAV CLv-R7, AAV CLv-R8, AAV CLv-R9, AAV CSp-1, AAV CSp-10, AAV CSp-11, AAV CSp-2, AAV CSp-3, AAV CSp-4, AAV CSp-6, AAV CSp-7, AAV CSp-8, AAV CSp-8.10, AAV CSp-8.2, AAV CSp-8.4, AAV CSp-8.5, AAV CSp-8.6, AAV CSp-8.7, AAV CSp-8.8, AAV CSp-8.9, AAV CSp-9, AAV.hu.48R3, AAV.VR-355, AAV3B, AAV4, AAV5, AAVF1/HSC1, AAVF11/HSC11, AAVF12/HSC12, AAVF13/HSC13, AAVF14/HSC14,

AAVF15/HSC15, AAVF16/HSC16, AAVF17/HSC17, AAVF2/HSC2, AAVF3/HSC3, AAVF4/HSC4, AAVF5/HSC5, AAVF6/HSC6, AAVF7/HSC7, AAVF8/HSC8, and/or AAVF9/HSC9 and variants thereof.

[066] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Publication No. US20030138772, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV1 (SEQ ID NO: 6 and 64 of US20030138772), AAV2 (SEQ ID NO: 7 and 70 of US20030138772), AAV3 (SEQ ID NO: 8 and 71 of US20030138772), AAV4 (SEQ ID NO: 63 of US20030138772), AAV5 (SEQ ID NO: 114 of US20030138772), AAV6 (SEQ ID NO: 65 of US20030138772), AAV7 (SEQ ID NO: 1-3 of US20030138772), AAV8 (SEQ ID NO: 4 and 95 of US20030138772), AAV9 (SEQ ID NO: 5 and 100 of US20030138772), AAV10 (SEQ ID NO: 117 of US20030138772), AAV11 (SEQ ID NO: 118 of US20030138772), AAV12 (SEQ ID NO: 119 of US20030138772), AAVrh10 (amino acids 1 to 738 of SEQ ID NO: 81 of US20030138772), AAV16.3 (US20030138772 SEQ ID NO: 10), AAV29.3/bb.1 (US20030138772 SEQ ID NO: 11), AAV29.4 (US20030138772 SEQ ID NO: 12), AAV29.5/bb.2 (US20030138772 SEQ ID NO: 13), AAV1.3 (US20030138772 SEQ ID NO: 14), AAV13.3 (US20030138772 SEQ ID NO: 15), AAV24.1 (US20030138772 SEQ ID NO: 16), AAV27.3 (US20030138772 SEQ ID NO: 17), AAV7.2 (US20030138772 SEQ ID NO: 18), AAVC1 (US20030138772 SEQ ID NO: 19), AAVC3 (US20030138772 SEQ ID NO: 20), AAVC5 (US20030138772 SEQ ID NO: 21), AAVF1 (US20030138772 SEQ ID NO: 22), AAVF3 (US20030138772 SEQ ID NO: 23), AAVF5 (US20030138772 SEQ ID NO: 24), AAVH6 (US20030138772 SEQ ID NO: 25), AAVH2 (US20030138772 SEQ ID NO: 26), AAV42-8 (US20030138772 SEQ ID NO: 27), AAV42-15 (US20030138772 SEQ ID NO: 28), AAV42-5b (US20030138772 SEQ ID NO: 29), AAV42-1b (US20030138772 SEQ ID NO: 30), AAV42-13 (US20030138772 SEQ ID NO: 31), AAV42-3a (US20030138772 SEQ ID NO: 32), AAV42-4 (US20030138772 SEQ ID NO: 33), AAV42-5a (US20030138772 SEQ ID NO: 34), AAV42-10 (US20030138772 SEQ ID NO: 35), AAV42-3b (US20030138772 SEQ ID NO: 36), AAV42-11 (US20030138772 SEQ ID NO: 37), AAV42-6b (US20030138772 SEQ ID NO: 38), AAV43-1 (US20030138772 SEQ ID NO: 39), AAV43-5 (US20030138772 SEQ ID NO: 40), AAV43-12 (US20030138772 SEQ ID NO: 41), AAV43-20 (US20030138772 SEQ ID NO: 42), AAV43-21 (US20030138772 SEQ ID NO: 43), AAV43-23 (US20030138772 SEQ ID NO: 44), AAV43-25 (US20030138772 SEQ ID NO: 45), AAV44.1 (US20030138772 SEQ ID NO: 46), AAV44.5 (US20030138772 SEQ ID NO: 47), AAV223.1 (US20030138772 SEQ ID NO: 48), AAV223.2 (US20030138772 SEQ ID NO: 49), AAV223.4 (US20030138772 SEQ ID NO: 50), AAV223.5 (US20030138772 SEQ ID NO: 51),

AAV223.6 (US20030138772 SEQ ID NO: 52), AAV223.7 (US20030138772 SEQ ID NO: 53), AAVA3.4 (US20030138772 SEQ ID NO: 54), AAVA3.5 (US20030138772 SEQ ID NO: 55), AAVA3.7 (US20030138772 SEQ ID NO: 56), AAVA3.3 (US20030138772 SEQ ID NO: 57), AAV42.12 (US20030138772 SEQ ID NO: 58), AAV44.2 (US20030138772 SEQ ID NO: 59), AAV42-2 (US20030138772 SEQ ID NO: 9), or variants thereof.

[067] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Publication No. US20150159173, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV2 (SEQ ID NO: 7 and 23 of US20150159173), rh20 (SEQ ID NO: 1 of US20150159173), rh32/33 (SEQ ID NO: 2 of US20150159173), rh39 (SEQ ID NO: 3, 20 and 36 of US20150159173), rh46 (SEQ ID NO: 4 and 22 of US20150159173), rh73 (SEQ ID NO: 5 of US20150159173), rh74 (SEQ ID NO: 6 of US20150159173), AAV6.1 (SEQ ID NO: 29 of US20150159173), rh.8 (SEQ ID NO: 41 of US20150159173), rh.48.1 (SEQ ID NO: 44 of US20150159173), hu.44 (SEQ ID NO: 45 of US20150159173), hu.29 (SEQ ID NO: 42 of US20150159173), hu.48 (SEQ ID NO: 38 of US20150159173), rh54 (SEQ ID NO: 49 of US20150159173), AAV2 (SEQ ID NO: 7 of US20150159173), cy.5 (SEQ ID NO: 8 and 24 of US20150159173), rh.10 (SEQ ID NO: 9 and 25 of US20150159173), rh.13 (SEQ ID NO: 10 and 26 of US20150159173), AAV1 (SEQ ID NO: 11 and 27 of US20150159173), AAV3 (SEQ ID NO: 12 and 28 of US20150159173), AAV6 (SEQ ID NO: 13 and 29 of US20150159173), AAV7 (SEQ ID NO: 14 and 30 of US20150159173), AAV8 (SEQ ID NO: 15 and 31 of US20150159173), hu.13 (SEQ ID NO: 16 and 32 of US20150159173), hu.26 (SEQ ID NO: 17 and 33 of US20150159173), hu.37 (SEQ ID NO: 18 and 34 of US20150159173), hu.53 (SEQ ID NO: 19 and 35 of US20150159173), rh.43 (SEQ ID NO: 21 and 37 of US20150159173), rh2 (SEQ ID NO: 39 of US20150159173), rh.37 (SEQ ID NO: 40 of US20150159173), rh.64 (SEQ ID NO: 43 of US20150159173), rh.48 (SEQ ID NO: 44 of US20150159173), ch.5 (SEQ ID NO 46 of US20150159173), rh.67 (SEQ ID NO: 47 of US20150159173), rh.58 (SEQ ID NO: 48 of US20150159173), or variants thereof including, but not limited to Cy5R1, Cy5R2, Cy5R3, Cy5R4, rh.13R, rh.37R2, rh.2R, rh.8R, rh.48.1, rh.48.2, rh.48.1.2, hu.44R1, hu.44R2, hu.44R3, hu.29R, ch.5R1, rh64R1, rh64R2, AAV6.2, AAV6.1, AAV6.12, hu.48R1, hu.48R2, and hu.48R3.

[068] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent No. US 7198951, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV9 (SEQ ID NO: 1-3 of US 7198951), AAV2 (SEQ ID NO: 4 of US 7198951), AAV1 (SEQ ID NO: 5 of US 7198951), AAV3 (SEQ ID NO: 6 of US 7198951), and AAV8 (SEQ ID NO: 7 of US7198951).

[069] In some embodiments, the AAV serotype may be, or have, a mutation in the AAV9 sequence as described by N Pulicherla *et al.* (Molecular Therapy 19(6):1070-1078 (2011), herein incorporated by reference in its entirety), such as but not limited to, AAV9.9, AAV9.11, AAV9.13, AAV9.16, AAV9.24, AAV9.45, AAV9.47, AAV9.61, AAV9.68, AAV9.84.

[070] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent No. US 6156303, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV3B (SEQ ID NO: 1 and 10 of US 6156303), AAV6 (SEQ ID NO: 2, 7 and 11 of US 6156303), AAV2 (SEQ ID NO: 3 and 8 of US 6156303), AAV3A (SEQ ID NO: 4 and 9, of US 6156303), or derivatives thereof.

[071] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Publication No. US20140359799, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV8 (SEQ ID NO: 1 of US20140359799), AAVDJ (SEQ ID NO: 2 and 3 of US20140359799), or variants thereof.

[072] In some embodiments, the serotype may be AAVDJ or a variant thereof, such as AAVDJ8 (or AAV-DJ8), as described by Grimm *et al.* (Journal of Virology 82(12): 5887-5911 (2008), herein incorporated by reference in its entirety). The amino acid sequence of AAVDJ8 may comprise two or more mutations in order to remove the heparin binding domain (HBD). As a non-limiting example, the AAV-DJ sequence described as SEQ ID NO: 1 in US Patent No. 7,588,772, the contents of which are herein incorporated by reference in their entirety, may comprise two mutations: (1) R587Q where arginine (R; Arg) at amino acid 587 is changed to glutamine (Q; Gln) and (2) R590T where arginine (R; Arg) at amino acid 590 is changed to threonine (T; Thr). As another non-limiting example, may comprise three mutations: (1) K406R where lysine (K; Lys) at amino acid 406 is changed to arginine (R; Arg), (2) R587Q where arginine (R; Arg) at amino acid 587 is changed to glutamine (Q; Gln) and (3) R590T where arginine (R; Arg) at amino acid 590 is changed to threonine (T; Thr).

[073] In some embodiments, the AAV serotype may be, or have, a sequence of AAV4 as described in International Publication No. WO1998011244, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to AAV4 (SEQ ID NO: 1-20 of WO1998011244).

[074] In some embodiments, the AAV serotype may be, or have, a mutation in the AAV2 sequence to generate AAV2G9 as described in International Publication No. WO2014144229 and herein incorporated by reference in its entirety.

[075] In some embodiments, the AAV serotype may be, or have, a sequence as described in International Publication No. WO2005033321, the contents of which are herein incorporated by

reference in their entirety, such as, but not limited to AAV3-3 (SEQ ID NO: 217 of WO2005033321), AAV1 (SEQ ID NO: 219 and 202 of WO2005033321), AAV106.1/hu.37 (SEQ ID No: 10 of WO2005033321), AAV114.3/hu.40 (SEQ ID No: 11 of WO2005033321), AAV127.2/hu.41 (SEQ ID NO:6 and 8 of WO2005033321), AAV128.3/hu.44 (SEQ ID No: 81 of WO2005033321), AAV130.4/hu.48 (SEQ ID NO: 78 of WO2005033321), AAV145.1/hu.53 (SEQ ID No: 176 and 177 of WO2005033321), AAV145.6/hu.56 (SEQ ID NO: 168 and 192 of WO2005033321), AAV16.12/hu.11 (SEQ ID NO: 153 and 57 of WO2005033321), AAV16.8/hu.10 (SEQ ID NO: 156 and 56 of WO2005033321), AAV161.10/hu.60 (SEQ ID No: 170 of WO2005033321), AAV161.6/hu.61 (SEQ ID No: 174 of WO2005033321), AAV1-7/rh.48 (SEQ ID NO: 32 of WO2005033321), AAV1-8/rh.49 (SEQ ID NOs: 103 and 25 of WO2005033321), AAV2 (SEQ ID NO: 211 and 221 of WO2005033321), AAV2-15/rh.62 (SEQ ID No: 33 and 114 of WO2005033321), AAV2-3/rh.61 (SEQ ID NO: 21 of WO2005033321), AAV2-4/rh.50 (SEQ ID No: 23 and 108 of WO2005033321), AAV2-5/rh.51 (SEQ ID NO: 104 and 22 of WO2005033321), AAV3.1/hu.6 (SEQ ID NO: 5 and 84 of WO2005033321), AAV3.1/hu.9 (SEQ ID NO: 155 and 58 of WO2005033321), AAV3-11/rh.53 (SEQ ID NO: 186 and 176 of WO2005033321), AAV3-3 (SEQ ID NO: 200 of WO2005033321), AAV33.12/hu.17 (SEQ ID NO:4 of WO2005033321), AAV33.4/hu.15 (SEQ ID No: 50 of WO2005033321), AAV33.8/hu.16 (SEQ ID No: 51 of WO2005033321), AAV3-9/rh.52 (SEQ ID NO: 96 and 18 of WO2005033321), AAV4-19/rh.55 (SEQ ID NO: 117 of WO2005033321), AAV4-4 (SEQ ID NO: 201 and 218 of WO2005033321), AAV4-9/rh.54 (SEQ ID NO: 116 of WO2005033321), AAV5 (SEQ ID NO: 199 and 216 of WO2005033321), AAV52.1/hu.20 (SEQ ID NO: 63 of WO2005033321), AAV52/hu.19 (SEQ ID NO: 133 of WO2005033321), AAV5-22/rh.58 (SEQ ID No: 27 of WO2005033321), AAV5-3/rh.57 (SEQ ID NO: 105 of WO2005033321), AAV5-3/rh.57 (SEQ ID No: 26 of WO2005033321), AAV58.2/hu.25 (SEQ ID No: 49 of WO2005033321), AAV6 (SEQ ID NO: 203 and 220 of WO2005033321), AAV7 (SEQ ID NO: 222 and 213 of WO2005033321), AAV7.3/hu.7 (SEQ ID No: 55 of WO2005033321), AAV8 (SEQ ID NO: 223 and 214 of WO2005033321), AAVH-1/hu.1 (SEQ ID No: 46 of WO2005033321), AAVH-5/hu.3 (SEQ ID No: 44 of WO2005033321), AAVhu.1 (SEQ ID NO: 144 of WO2005033321), AAVhu.10 (SEQ ID NO: 156 of WO2005033321), AAVhu.11 (SEQ ID NO: 153 of WO2005033321), AAVhu.12 (WO2005033321 SEQ ID NO: 59), AAVhu.13 (SEQ ID NO: 129 of WO2005033321), AAVhu.14/AAV9 (SEQ ID NO: 123 and 3 of WO2005033321), AAVhu.15 (SEQ ID NO: 147 of WO2005033321), AAVhu.16 (SEQ ID NO: 148 of WO2005033321), AAVhu.17 (SEQ ID NO: 83 of WO2005033321), AAVhu.18 (SEQ ID NO: 149 of WO2005033321), AAVhu.19 (SEQ ID NO: 133 of WO2005033321), AAVhu.2

(SEQ ID NO: 143 of WO2005033321), AAVhu.20 (SEQ ID NO: 134 of WO2005033321), AAVhu.21 (SEQ ID NO: 135 of WO2005033321), AAVhu.22 (SEQ ID NO: 138 of WO2005033321), AAVhu.23.2 (SEQ ID NO: 137 of WO2005033321), AAVhu.24 (SEQ ID NO: 136 of WO2005033321), AAVhu.25 (SEQ ID NO: 146 of WO2005033321), AAVhu.27 (SEQ ID NO: 140 of WO2005033321), AAVhu.29 (SEQ ID NO: 132 of WO2005033321), AAVhu.3 (SEQ ID NO: 145 of WO2005033321), AAVhu.31 (SEQ ID NO: 121 of WO2005033321), AAVhu.32 (SEQ ID NO: 122 of WO2005033321), AAVhu.34 (SEQ ID NO: 125 of WO2005033321), AAVhu.35 (SEQ ID NO: 164 of WO2005033321), AAVhu.37 (SEQ ID NO: 88 of WO2005033321), AAVhu.39 (SEQ ID NO: 102 of WO2005033321), AAVhu.4 (SEQ ID NO: 141 of WO2005033321), AAVhu.40 (SEQ ID NO: 87 of WO2005033321), AAVhu.41 (SEQ ID NO: 91 of WO2005033321), AAVhu.42 (SEQ ID NO: 85 of WO2005033321), AAVhu.43 (SEQ ID NO: 160 of WO2005033321), AAVhu.44 (SEQ ID NO: 144 of WO2005033321), AAVhu.45 (SEQ ID NO: 127 of WO2005033321), AAVhu.46 (SEQ ID NO: 159 of WO2005033321), AAVhu.47 (SEQ ID NO: 128 of WO2005033321), AAVhu.48 (SEQ ID NO: 157 of WO2005033321), AAVhu.49 (SEQ ID NO: 189 of WO2005033321), AAVhu.51 (SEQ ID NO: 190 of WO2005033321), AAVhu.52 (SEQ ID NO: 191 of WO2005033321), AAVhu.53 (SEQ ID NO: 186 of WO2005033321), AAVhu.54 (SEQ ID NO: 188 of WO2005033321), AAVhu.55 (SEQ ID NO: 187 of WO2005033321), AAVhu.56 (SEQ ID NO: 192 of WO2005033321), AAVhu.57 (SEQ ID NO: 193 of WO2005033321), AAVhu.58 (SEQ ID NO: 194 of WO2005033321), AAVhu.6 (SEQ ID NO: 84 of WO2005033321), AAVhu.60 (SEQ ID NO: 184 of WO2005033321), AAVhu.61 (SEQ ID NO: 185 of WO2005033321), AAVhu.63 (SEQ ID NO: 195 of WO2005033321), AAVhu.64 (SEQ ID NO: 196 of WO2005033321), AAVhu.66 (SEQ ID NO: 197 of WO2005033321), AAVhu.67 (SEQ ID NO: 198 of WO2005033321), AAVhu.7 (SEQ ID NO: 150 of WO2005033321), AAVhu.8 (WO2005033321 SEQ ID NO: 12), AAVhu.9 (SEQ ID NO: 155 of WO2005033321), AAVLG-10/rh.40 (SEQ ID No: 14 of WO2005033321), AAVLG-4/rh.38 (SEQ ID NO: 86 of WO2005033321), AAVLG-4/rh.38 (SEQ ID No: 7 of WO2005033321), AAVN721-8/rh.43 (SEQ ID NO: 163 of WO2005033321), AAVN721-8/rh.43 (SEQ ID No: 43 of WO2005033321), AAVpi.1 (WO2005033321 SEQ ID NO: 28), AAVpi.2 (WO2005033321 SEQ ID NO: 30), AAVpi.3 (WO2005033321 SEQ ID NO: 29), AAVrh.38 (SEQ ID NO: 86 of WO2005033321), AAVrh.40 (SEQ ID NO: 92 of WO2005033321), AAVrh.43 (SEQ ID NO: 163 of WO2005033321), AAVrh.44 (WO2005033321 SEQ ID NO: 34), AAVrh.45 (WO2005033321 SEQ ID NO: 41), AAVrh.47 (WO2005033321 SEQ ID NO: 38), AAVrh.48 (SEQ ID NO: 115 of WO2005033321), AAVrh.49 (SEQ ID NO: 103 of WO2005033321),

AAVrh.50 (SEQ ID NO: 108 of WO2005033321), AAVrh.51 (SEQ ID NO: 104 of WO2005033321), AAVrh.52 (SEQ ID NO: 96 of WO2005033321), AAVrh.53 (SEQ ID NO: 97 of WO2005033321), AAVrh.55 (WO2005033321 SEQ ID NO: 37), AAVrh.56 (SEQ ID NO: 152 of WO2005033321), AAVrh.57 (SEQ ID NO: 105 of WO2005033321), AAVrh.58 (SEQ ID NO: 106 of WO2005033321), AAVrh.59 (WO2005033321 SEQ ID NO: 42), AAVrh.60 (WO2005033321 SEQ ID NO: 31), AAVrh.61 (SEQ ID NO: 107 of WO2005033321), AAVrh.62 (SEQ ID NO: 114 of WO2005033321), AAVrh.64 (SEQ ID NO: 99 of WO2005033321), AAVrh.65 (WO2005033321 SEQ ID NO: 35), AAVrh.68 (WO2005033321 SEQ ID NO: 16), AAVrh.69 (WO2005033321 SEQ ID NO: 39), AAVrh.70 (WO2005033321 SEQ ID NO: 20), AAVrh.72 (WO2005033321 SEQ ID NO: 9), or variants thereof including, but not limited to, AAVcy.2, AAVcy.3, AAVcy.4, AAVcy.5, AAVcy.6, AAVrh.12, AAVrh.17, AAVrh.18, AAVrh.19, AAVrh.21, AAVrh.22, AAVrh.23, AAVrh.24, AAVrh.25, AAVrh.25/42 15, AAVrh.31, AAVrh.32, AAVrh.33, AAVrh.34, AAVrh.35, AAVrh.36, AAVrh.37, AAVrh.14. Non limiting examples of variants include SEQ ID NO: 13, 15, 17, 19, 24, 36, 40, 45, 47, 48, 51-54, 60-62, 64-77, 79, 80, 82, 89, 90, 93-95, 98, 100, 101, , 109-113, 118-120, 124, 126, 131, 139, 142, 151,154, 158, 161, 162, 165-183, 202, 204-212, 215, 219, 224-236, of WO2005033321, the contents of which are herein incorporated by reference in their entirety.

[076] In some embodiments, the AAV serotype may be, or have, a sequence as described in International Publication No. WO2015168666, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAVrh8R (SEQ ID NO: 9 of WO2015168666), AAVrh8R A586R mutant (SEQ ID NO: 10 of WO2015168666), AAVrh8R R533A mutant (SEQ ID NO: 11 of WO2015168666), or variants thereof.

[077] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent No. US9233131, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAVhE1.1 (SEQ ID NO:44 of US9233131), AAVhEr1.5 (SEQ ID NO:45 of US9233131), AAVhEr1.14 (SEQ ID NO:46 of US9233131), AAVhEr1.8 (SEQ ID NO:47 of US9233131), AAVhEr1.16 (SEQ ID NO:48 of US9233131), AAVhEr1.18 (SEQ ID NO:49 of US9233131), AAVhEr1.35 (SEQ ID NO:50 of US9233131), AAVhEr1.7 (SEQ ID NO:51 of US9233131), AAVhEr1.36 (SEQ ID NO:52 of US9233131), AAVhEr2.29 (SEQ ID NO:53 of US9233131), AAVhEr2.4 (SEQ ID NO:54 of US9233131), AAVhEr2.16 (SEQ ID NO:55 of US9233131), AAVhEr2.30 (SEQ ID NO:56 of US9233131), AAVhEr2.31 (SEQ ID NO:58 of US9233131), AAVhEr2.36 (SEQ ID NO:57 of US9233131), AAVhEr1.23 (SEQ ID NO:53 of US9233131), AAVhEr3.1 (SEQ ID NO:59 of US9233131),

AAV2.5T (SEQ ID NO:42 of US9233131), or variants thereof.

[078] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent Publication No. US20150376607, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV-PAEC (SEQ ID NO:1 of US20150376607), AAV-LK01 (SEQ ID NO:2 of US20150376607), AAV-LK02 (SEQ ID NO:3 of US20150376607), AAV-LK03 (SEQ ID NO:4 of US20150376607), AAV-LK04 (SEQ ID NO:5 of US20150376607), AAV-LK05 (SEQ ID NO:6 of US20150376607), AAV-LK06 (SEQ ID NO:7 of US20150376607), AAV-LK07 (SEQ ID NO:8 of US20150376607), AAV-LK08 (SEQ ID NO:9 of US20150376607), AAV-LK09 (SEQ ID NO:10 of US20150376607), AAV-LK10 (SEQ ID NO:11 of US20150376607), AAV-LK11 (SEQ ID NO:12 of US20150376607), AAV-LK12 (SEQ ID NO:13 of US20150376607), AAV-LK13 (SEQ ID NO:14 of US20150376607), AAV-LK14 (SEQ ID NO:15 of US20150376607), AAV-LK15 (SEQ ID NO:16 of US20150376607), AAV-LK16 (SEQ ID NO:17 of US20150376607), AAV-LK17 (SEQ ID NO:18 of US20150376607), AAV-LK18 (SEQ ID NO:19 of US20150376607), AAV-LK19 (SEQ ID NO:20 of US20150376607), AAV-PAEC2 (SEQ ID NO:21 of US20150376607), AAV-PAEC4 (SEQ ID NO:22 of US20150376607), AAV-PAEC6 (SEQ ID NO:23 of US20150376607), AAV-PAEC7 (SEQ ID NO:24 of US20150376607), AAV-PAEC8 (SEQ ID NO:25 of US20150376607), AAV-PAEC11 (SEQ ID NO:26 of US20150376607), AAV-PAEC12 (SEQ ID NO:27, of US20150376607), or variants thereof.

[079] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent No. US9163261, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV-2-pre-miRNA-101 (SEQ ID NO: 1 US9163261), or variants thereof.

[080] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent Publication No. US20150376240, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV-8h (SEQ ID NO: 6 of US20150376240), AAV-8b (SEQ ID NO: 5 of US20150376240), AAV-h (SEQ ID NO: 2 of US20150376240), AAV-b (SEQ ID NO: 1 of US20150376240), or variants thereof.

[081] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent Publication No. US20160017295, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV SM 10-2 (SEQ ID NO: 22 of US20160017295), AAV Shuffle 100-1 (SEQ ID NO: 23 of US20160017295), AAV Shuffle 100-3 (SEQ ID NO: 24 of US20160017295), AAV Shuffle 100-7 (SEQ ID NO: 25 of US20160017295), AAV Shuffle 10-2 (SEQ ID NO: 34 of US20160017295), AAV Shuffle 10-6

(SEQ ID NO: 35 of US20160017295), AAV Shuffle 10-8 (SEQ ID NO: 36 of US20160017295), AAV Shuffle 100-2 (SEQ ID NO: 37 of US20160017295), AAV SM 10-1 (SEQ ID NO: 38 of US20160017295), AAV SM 10-8 (SEQ ID NO: 39 of US20160017295), AAV SM 100-3 (SEQ ID NO: 40 of US20160017295), AAV SM 100-10 (SEQ ID NO: 41 of US20160017295), or variants thereof.

[082] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent Publication No. US20150238550, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, BNP61 AAV (SEQ ID NO: 1 of US20150238550), BNP62 AAV (SEQ ID NO: 3 of US20150238550), BNP63 AAV (SEQ ID NO: 4 of US20150238550), or variants thereof.

[083] In some embodiments, the AAV serotype may be or may have a sequence as described in United States Patent Publication No. US20150315612, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAVrh.50 (SEQ ID NO: 108 of US20150315612), AAVrh.43 (SEQ ID NO: 163 of US20150315612), AAVrh.62 (SEQ ID NO: 114 of US20150315612), AAVrh.48 (SEQ ID NO: 115 of US20150315612), AAVhu.19 (SEQ ID NO: 133 of US20150315612), AAVhu.11 (SEQ ID NO: 153 of US20150315612), AAVhu.53 (SEQ ID NO: 186 of US20150315612), AAV4-8/rh.64 (SEQ ID No: 15 of US20150315612), AAVLG-9/hu.39 (SEQ ID No: 24 of US20150315612), AAV54.5/hu.23 (SEQ ID No: 60 of US20150315612), AAV54.2/hu.22 (SEQ ID No: 67 of US20150315612), AAV54.7/hu.24 (SEQ ID No: 66 of US20150315612), AAV54.1/hu.21 (SEQ ID No: 65 of US20150315612), AAV54.4R/hu.27 (SEQ ID No: 64 of US20150315612), AAV46.2/hu.28 (SEQ ID No: 68 of US20150315612), AAV46.6/hu.29 (SEQ ID No: 69 of US20150315612), AAV128.1/hu.43 (SEQ ID No: 80 of US20150315612), or variants thereof.

[084] In some embodiments, the AAV serotype may be, or have, a sequence as described in International Publication No. WO2015121501, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, true type AAV (ttAAV) (SEQ ID NO: 2 of WO2015121501), “UPenn AAV10” (SEQ ID NO: 8 of WO2015121501), “Japanese AAV10” (SEQ ID NO: 9 of WO2015121501), or variants thereof.

[085] According to the present disclosure, AAV capsid serotype selection or use may be from a variety of species. In some embodiments, the AAV may be an avian AAV (AAAV). The AAAV serotype may be, or have, a sequence as described in United States Patent No. US 9238800, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAAV (SEQ ID NO: 1, 2, 4, 6, 8, 10, 12, and 14 of US 9,238,800), or variants thereof.

[086] In some embodiments, the AAV may be a bovine AAV (BAAV). The BAAV serotype may be, or have, a sequence as described in United States Patent No. US 9,193,769, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, BAAV (SEQ ID NO: 1 and 6 of US 9193769), or variants thereof. The BAAV serotype may be or have a sequence as described in United States Patent No. US7427396, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, BAAV (SEQ ID NO: 5 and 6 of US7427396), or variants thereof.

[087] In some embodiments, the AAV may be a caprine AAV. The caprine AAV serotype may be, or have, a sequence as described in United States Patent No. US7427396, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, caprine AAV (SEQ ID NO: 3 of US7427396), or variants thereof.

[088] In other embodiments the AAV may be engineered as a hybrid AAV from two or more parental serotypes. In some embodiments, the AAV may be AAV2G9 which comprises sequences from AAV2 and AAV9. The AAV2G9 AAV serotype may be, or have, a sequence as described in United States Patent Publication No. US20160017005, the contents of which are herein incorporated by reference in their entirety.

[089] In some embodiments, the AAV may be a serotype generated by the AAV9 capsid library with mutations in amino acids 390-627 (VP1 numbering) as described by Pulicherla *et al.* (Molecular Therapy 19(6):1070-1078 (2011), the contents of which are herein incorporated by reference in their entirety. The serotype and corresponding nucleotide and amino acid substitutions may be, but is not limited to, AAV9.1 (G1594C; D532H), AAV6.2 (T1418A and T1436X; V473D and I479K), AAV9.3 (T1238A; F413Y), AAV9.4 (T1250C and A1617T; F417S), AAV9.5 (A1235G, A1314T, A1642G, C1760T; Q412R, T548A, A587V), AAV9.6 (T1231A; F411I), AAV9.9 (G1203A, G1785T; W595C), AAV9.10 (A1500G, T1676C; M559T), AAV9.11 (A1425T, A1702C, A1769T; T568P, Q590L), AAV9.13 (A1369C, A1720T; N457H, T574S), AAV9.14 (T1340A, T1362C, T1560C, G1713A; L447H), AAV9.16 (A1775T; Q592L), AAV9.24 (T1507C, T1521G; W503R), AAV9.26 (A1337G, A1769C; Y446C, Q590P), AAV9.33 (A1667C; D556A), AAV9.34 (A1534G, C1794T; N512D), AAV9.35 (A1289T, T1450A, C1494T, A1515T, C1794A, G1816A; Q430L, Y484N, N98K, V606I), AAV9.40 (A1694T, E565V), AAV9.41 (A1348T, T1362C; T450S), AAV9.44 (A1684C, A1701T, A1737G; N562H, K567N), AAV9.45 (A1492T, C1804T; N498Y, L602F), AAV9.46 (G1441C, T1525C, T1549G; G481R, W509R, L517V), 9.47 (G1241A, G1358A, A1669G, C1745T; S414N, G453D, K557E, T582I), AAV9.48 (C1445T, A1736T; P482L, Q579L), AAV9.50 (A1638T, C1683T, T1805A; Q546H, L602H), AAV9.53 (G1301A, A1405C,

C1664T, G1811T; R134Q, S469R, A555V, G604V), AAV9.54 (C1531A, T1609A; L511I, L537M), AAV9.55 (T1605A; F535L), AAV9.58 (C1475T, C1579A; T492I, H527N), AAV.59 (T1336C; Y446H), AAV9.61 (A1493T; N498I), AAV9.64 (C1531A, A1617T; L511I), AAV9.65 (C1335T, T1530C, C1568A; A523D), AAV9.68 (C1510A; P504T), AAV9.80 (G1441A,,G481R), AAV9.83 (C1402A, A1500T; P468T, E500D), AAV9.87 (T1464C, T1468C; S490P), AAV9.90 (A1196T; Y399F), AAV9.91 (T1316G, A1583T, C1782G, T1806C; L439R, K528I), AAV9.93 (A1273G, A1421G, A1638C, C1712T, G1732A, A1744T, A1832T; S425G, Q474R, Q546H, P571L, G578R, T582S, D611V), AAV9.94 (A1675T; M559L) and AAV9.95 (T1605A; F535L).

[090] In some embodiments, the AAV serotype may be, or have, a sequence as described in International Publication No. WO2016049230, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to AAVF1/HSC1 (SEQ ID NO: 2 and 20 of WO2016049230), AAVF2/HSC2 (SEQ ID NO: 3 and 21 of WO2016049230), AAVF3/HSC3 (SEQ ID NO: 5 and 22 of WO2016049230), AAVF4/HSC4 (SEQ ID NO: 6 and 23 of WO2016049230), AAVF5/HSC5 (SEQ ID NO: 11 and 25 of WO2016049230), AAVF6/HSC6 (SEQ ID NO: 7 and 24 of WO2016049230), AAVF7/HSC7 (SEQ ID NO: 8 and 27 of WO2016049230), AAVF8/HSC8 (SEQ ID NO: 9 and 28 of WO2016049230), AAVF9/HSC9 (SEQ ID NO: 10 and 29 of WO2016049230), AAVF11/HSC11 (SEQ ID NO: 4 and 26 of WO2016049230), AAVF12/HSC12 (SEQ ID NO: 12 and 30 of WO2016049230), AAVF13/HSC13 (SEQ ID NO: 14 and 31 of WO2016049230), AAVF14/HSC14 (SEQ ID NO: 15 and 32 of WO2016049230), AAVF15/HSC15 (SEQ ID NO: 16 and 33 of WO2016049230), AAVF16/HSC16 (SEQ ID NO: 17 and 34 of WO2016049230), AAVF17/HSC17 (SEQ ID NO: 13 and 35 of WO2016049230), or variants or derivatives thereof.

[091] In some embodiments, the AAV serotype may be, or have, a sequence as described in United States Patent No. US 8734809, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV CBr-E1 (SEQ ID NO: 13 and 87 of US8734809), AAV CBr-E2 (SEQ ID NO: 14 and 88 of US8734809), AAV CBr-E3 (SEQ ID NO: 15 and 89 of US8734809), AAV CBr-E4 (SEQ ID NO: 16 and 90 of US8734809), AAV CBr-E5 (SEQ ID NO: 17 and 91 of US8734809), AAV CBr-e5 (SEQ ID NO: 18 and 92 of US8734809), AAV CBr-E6 (SEQ ID NO: 19 and 93 of US8734809), AAV CBr-E7 (SEQ ID NO: 20 and 94 of US8734809), AAV CBr-E8 (SEQ ID NO: 21 and 95 of US8734809), AAV CLv-D1 (SEQ ID NO: 22 and 96 of US8734809), AAV CLv-D2 (SEQ ID NO: 23 and 97 of US8734809), AAV CLv-D3 (SEQ ID NO: 24 and 98 of US8734809), AAV CLv-D4 (SEQ ID NO: 25 and 99 of US8734809), AAV CLv-D5 (SEQ ID NO: 26 and 100 of US8734809), AAV

CLv-D6 (SEQ ID NO: 27 and 101 of US8734809), AAV CLv-D7 (SEQ ID NO: 28 and 102 of US8734809), AAV CLv-D8 (SEQ ID NO: 29 and 103 of US8734809), AAV CLv-E1 (SEQ ID NO: 13 and 87 of US8734809), AAV CLv-R1 (SEQ ID NO: 30 and 104 of US8734809), AAV CLv-R2 (SEQ ID NO: 31 and 105 of US8734809), AAV CLv-R3 (SEQ ID NO: 32 and 106 of US8734809), AAV CLv-R4 (SEQ ID NO: 33 and 107 of US8734809), AAV CLv-R5 (SEQ ID NO: 34 and 108 of US8734809), AAV CLv-R6 (SEQ ID NO: 35 and 109 of US8734809), AAV CLv-R7 (SEQ ID NO: 36 and 110 of US8734809), AAV CLv-R8 (SEQ ID NO: X and X of US8734809), AAV CLv-R9 (SEQ ID NO: X and X of US8734809), AAV CLg-F1 (SEQ ID NO: 39 and 113 of US8734809), AAV CLg-F2 (SEQ ID NO: 40 and 114 of US8734809), AAV CLg-F3 (SEQ ID NO: 41 and 115 of US8734809), AAV CLg-F4 (SEQ ID NO: 42 and 116 of US8734809), AAV CLg-F5 (SEQ ID NO: 43 and 117 of US8734809), AAV CLg-F6 (SEQ ID NO: 43 and 117 of US8734809), AAV CLg-F7 (SEQ ID NO: 44 and 118 of US8734809), AAV CLg-F8 (SEQ ID NO: 43 and 117 of US8734809), AAV CSp-1 (SEQ ID NO: 45 and 119 of US8734809), AAV CSp-10 (SEQ ID NO: 46 and 120 of US8734809), AAV CSp-11 (SEQ ID NO: 47 and 121 of US8734809), AAV CSp-2 (SEQ ID NO: 48 and 122 of US8734809), AAV CSp-3 (SEQ ID NO: 49 and 123 of US8734809), AAV CSp-4 (SEQ ID NO: 50 and 124 of US8734809), AAV CSp-6 (SEQ ID NO: 51 and 125 of US8734809), AAV CSp-7 (SEQ ID NO: 52 and 126 of US8734809), AAV CSp-8 (SEQ ID NO: 53 and 127 of US8734809), AAV CSp-9 (SEQ ID NO: 54 and 128 of US8734809), AAV CHt-2 (SEQ ID NO: 55 and 129 of US8734809), AAV CHt-3 (SEQ ID NO: 56 and 130 of US8734809), AAV CKd-1 (SEQ ID NO: 57 and 131 of US8734809), AAV CKd-10 (SEQ ID NO: 58 and 132 of US8734809), AAV CKd-2 (SEQ ID NO: 59 and 133 of US8734809), AAV CKd-3 (SEQ ID NO: 60 and 134 of US8734809), AAV CKd-4 (SEQ ID NO: 61 and 135 of US8734809), AAV CKd-6 (SEQ ID NO: 62 and 136 of US8734809), AAV CKd-7 (SEQ ID NO: 63 and 137 of US8734809), AAV CKd-8 (SEQ ID NO: 64 and 138 of US8734809), AAV CLv-1 (SEQ ID NO: 35 and 139 of US8734809), AAV CLv-12 (SEQ ID NO: 66 and 140 of US8734809), AAV CLv-13 (SEQ ID NO: 67 and 141 of US8734809), AAV CLv-2 (SEQ ID NO: 68 and 142 of US8734809), AAV CLv-3 (SEQ ID NO: 69 and 143 of US8734809), AAV CLv-4 (SEQ ID NO: 70 and 144 of US8734809), AAV CLv-6 (SEQ ID NO: 71 and 145 of US8734809), AAV CLv-8 (SEQ ID NO: 72 and 146 of US8734809), AAV CKd-B1 (SEQ ID NO: 73 and 147 of US8734809), AAV CKd-B2 (SEQ ID NO: 74 and 148 of US8734809), AAV CKd-B3 (SEQ ID NO: 75 and 149 of US8734809), AAV CKd-B4 (SEQ ID NO: 76 and 150 of US8734809), AAV CKd-B5 (SEQ ID NO: 77 and 151 of US8734809), AAV CKd-B6 (SEQ ID NO: 78 and 152 of US8734809), AAV CKd-B7 (SEQ ID NO: 79 and 153 of US8734809), AAV CKd-B8 (SEQ ID NO: 80 and 154 of

US8734809), AAV CKd-H1 (SEQ ID NO: 81 and 155 of US8734809), AAV CKd-H2 (SEQ ID NO: 82 and 156 of US8734809), AAV CKd-H3 (SEQ ID NO: 83 and 157 of US8734809), AAV CKd-H4 (SEQ ID NO: 84 and 158 of US8734809), AAV CKd-H5 (SEQ ID NO: 85 and 159 of US8734809), AAV CKd-H6 (SEQ ID NO: 77 and 151 of US8734809), AAV CHt-1 (SEQ ID NO: 86 and 160 of US8734809), AAV CLv1-1 (SEQ ID NO: 171 of US8734809), AAV CLv1-2 (SEQ ID NO: 172 of US8734809), AAV CLv1-3 (SEQ ID NO: 173 of US8734809), AAV CLv1-4 (SEQ ID NO: 174 of US8734809), AAV Clv1-7 (SEQ ID NO: 175 of US8734809), AAV Clv1-8 (SEQ ID NO: 176 of US8734809), AAV Clv1-9 (SEQ ID NO: 177 of US8734809), AAV Clv1-10 (SEQ ID NO: 178 of US8734809), AAV.VR-355 (SEQ ID NO: 181 of US8734809), AAV.hu.48R3 (SEQ ID NO: 183 of US8734809), or variants or derivatives thereof.

[092] In some embodiments, the AAV serotype may be, or have, a sequence as described in International Publication No. WO2016065001, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to AAV CHt-P2 (SEQ ID NO: 1 and 51 of WO2016065001), AAV CHt-P5 (SEQ ID NO: 2 and 52 of WO2016065001), AAV CHt-P9 (SEQ ID NO: 3 and 53 of WO2016065001), AAV CBr-7.1 (SEQ ID NO: 4 and 54 of WO2016065001), AAV CBr-7.2 (SEQ ID NO: 5 and 55 of WO2016065001), AAV CBr-7.3 (SEQ ID NO: 6 and 56 of WO2016065001), AAV CBr-7.4 (SEQ ID NO: 7 and 57 of WO2016065001), AAV CBr-7.5 (SEQ ID NO: 8 and 58 of WO2016065001), AAV CBr-7.7 (SEQ ID NO: 9 and 59 of WO2016065001), AAV CBr-7.8 (SEQ ID NO: 10 and 60 of WO2016065001), AAV CBr-7.10 (SEQ ID NO: 11 and 61 of WO2016065001), AAV CKd-N3 (SEQ ID NO: 12 and 62 of WO2016065001), AAV CKd-N4 (SEQ ID NO: 13 and 63 of WO2016065001), AAV CKd-N9 (SEQ ID NO: 14 and 64 of WO2016065001), AAV CLv-L4 (SEQ ID NO: 15 and 65 of WO2016065001), AAV CLv-L5 (SEQ ID NO: 16 and 66 of WO2016065001), AAV CLv-L6 (SEQ ID NO: 17 and 67 of WO2016065001), AAV CLv-K1 (SEQ ID NO: 18 and 68 of WO2016065001), AAV CLv-K3 (SEQ ID NO: 19 and 69 of WO2016065001), AAV CLv-K6 (SEQ ID NO: 20 and 70 of WO2016065001), AAV CLv-M1 (SEQ ID NO: 21 and 71 of WO2016065001), AAV CLv-M11 (SEQ ID NO: 22 and 72 of WO2016065001), AAV CLv-M2 (SEQ ID NO: 23 and 73 of WO2016065001), AAV CLv-M5 (SEQ ID NO: 24 and 74 of WO2016065001), AAV CLv-M6 (SEQ ID NO: 25 and 75 of WO2016065001), AAV CLv-M7 (SEQ ID NO: 26 and 76 of WO2016065001), AAV CLv-M8 (SEQ ID NO: 27 and 77 of WO2016065001), AAV CLv-M9 (SEQ ID NO: 28 and 78 of WO2016065001), AAV CHt-P1 (SEQ ID NO: 29 and 79 of WO2016065001), AAV CHt-P6 (SEQ ID NO: 30 and 80 of WO2016065001), AAV CHt-P8 (SEQ ID NO: 31 and 81 of

WO2016065001), AAV CHt-6.1 (SEQ ID NO: 32 and 82 of WO2016065001), AAV CHt-6.10 (SEQ ID NO: 33 and 83 of WO2016065001), AAV CHt-6.5 (SEQ ID NO: 34 and 84 of WO2016065001), AAV CHt-6.6 (SEQ ID NO: 35 and 85 of WO2016065001), AAV CHt-6.7 (SEQ ID NO: 36 and 86 of WO2016065001), AAV CHt-6.8 (SEQ ID NO: 37 and 87 of WO2016065001), AAV CSp-8.10 (SEQ ID NO: 38 and 88 of WO2016065001), AAV CSp-8.2 (SEQ ID NO: 39 and 89 of WO2016065001), AAV CSp-8.4 (SEQ ID NO: 40 and 90 of WO2016065001), AAV CSp-8.5 (SEQ ID NO: 41 and 91 of WO2016065001), AAV CSp-8.6 (SEQ ID NO: 42 and 92 of WO2016065001), AAV CSp-8.7 (SEQ ID NO: 43 and 93 of WO2016065001), AAV CSp-8.8 (SEQ ID NO: 44 and 94 of WO2016065001), AAV CSp-8.9 (SEQ ID NO: 45 and 95 of WO2016065001), AAV CBr-B7.3 (SEQ ID NO: 46 and 96 of WO2016065001), AAV CBr-B7.4 (SEQ ID NO: 47 and 97 of WO2016065001), AAV3B (SEQ ID NO: 48 and 98 of WO2016065001), AAV4 (SEQ ID NO: 49 and 99 of WO2016065001), AAV5 (SEQ ID NO: 50 and 100 of WO2016065001), or variants or derivatives thereof.

[093] In some embodiments, the AAV particle may have, or may be a serotype selected from any of those found in Table 1.

[094] In some embodiments, the AAV capsid may comprise a sequence, fragment or variant thereof, of any of the sequences in Table 1.

[095] In some embodiments, the AAV capsid may be encoded by a sequence, fragment or variant as described in Table 1.

[096] In any of the DNA and RNA sequences referenced and/or described herein, the single letter symbol has the following description: A for adenine; C for cytosine; G for guanine; T for thymine; U for Uracil; W for weak bases such as adenine or thymine; S for strong nucleotides such as cytosine and guanine; M for amino nucleotides such as adenine and cytosine; K for keto nucleotides such as guanine and thymine; R for purines adenine and guanine; Y for pyrimidine cytosine and thymine; B for any base that is not A (*e.g.*, cytosine, guanine, and thymine); D for any base that is not C (*e.g.*, adenine, guanine, and thymine); H for any base that is not G (*e.g.*, adenine, cytosine, and thymine); V for any base that is not T (*e.g.*, adenine, cytosine, and guanine); N for any nucleotide (which is not a gap); and Z is for zero.

[097] In any of the amino acid sequences referenced and/or described herein, the single letter symbol has the following description: G (Gly) for Glycine; A (Ala) for Alanine; L (Leu) for Leucine; M (Met) for Methionine; F (Phe) for Phenylalanine; W (Trp) for Tryptophan; K (Lys) for Lysine; Q (Gln) for Glutamine; E (Glu) for Glutamic Acid; S (Ser) for Serine; P (Pro) for Proline; V (Val) for Valine; I (Ile) for Isoleucine; C (Cys) for Cysteine; Y (Tyr) for Tyrosine; H (His) for Histidine; R (Arg) for Arginine; N (Asn) for Asparagine; D (Asp) for Aspartic Acid;

T (Thr) for Threonine; B (Asx) for Aspartic acid or Asparagine; J (Xle) for Leucine or Isoleucine; O (Pyl) for Pyrrolysine; U (Sec) for Selenocysteine; X (Xaa) for any amino acid; and Z (Glx) for Glutamine or Glutamic acid.

Table 1. AAV Serotypes

Serotype	SEQ ID NO:	Reference Information
VOY101	1	-
VOY101	2	-
VOY201	3	-
PHP.N/PHP.B-DGT	4	WO2017100671 SEQ ID NO: 46
AAVPHP.B or G2B-26	5	WO2015038958 SEQ ID NO: 8 and 13
AAVPHP.B	6	WO2015038958 SEQ ID NO: 9
AAV5	102	US7427396 SEQ ID NO: 1
AAV5	103	US20030138772 SEQ ID NO: 114
AAV5	104	US20160017295 SEQ ID NO: 5, US7427396 SEQ ID NO: 2, US20150315612 SEQ ID NO: 216
AAV5	105	US20150315612 SEQ ID NO: 199
AAV9	132	US20030138772 SEQ ID NO: 5
AAV9	133	US7198951 SEQ ID NO: 1
AAV9	134	US20160017295 SEQ ID NO: 9
AAV9	135	US20030138772 SEQ ID NO: 100, US7198951 SEQ ID NO: 2
AAV9	136	US7198951 SEQ ID NO: 3
AAV9 (AAVhu.14)	137	US7906111 SEQ ID NO: 3; WO2015038958 SEQ ID NO: 11
AAV9 (AAVhu.14)	138	US7906111 SEQ ID NO: 123; WO2015038958 SEQ ID NO: 2
AAVrh.10	399	US20150159173 SEQ ID NO: 9
AAVrh.10	400	US20150159173 SEQ ID NO: 25
AAV5	878	WO2016065001 SEQ ID NO: 100
AAV9	996	WO2016073739A1 SEQ ID NO: 3
AAV2	1260	WO2016134375A1 SEQ ID NO: 9
AAV2	1261	WO2016134375A1 SEQ ID NO: 10

[098] In some embodiments, the AAV serotype may be, or may have a sequence as described in International Patent Publication WO2015038958, the contents of which are herein incorporated by reference in their entirety, such as, but not limited to, AAV9 (SEQ ID NO: 11 and 2 of WO2015038958 or SEQ ID NO: 137 and 138 respectively herein), PHP.B (SEQ ID NO: 8 and 9 of WO2015038958, herein SEQ ID NO: 5 and 6), G2B-13 (SEQ ID NO: 12 of WO2015038958, herein SEQ ID NO: 7), G2B-26 (SEQ ID NO: 13 of WO2015038958, herein SEQ ID NO: 5), TH1.1-32 (SEQ ID NO: 14 of WO2015038958, herein SEQ ID NO: 8), TH1.1-35 (SEQ ID NO: 15 of WO2015038958, herein SEQ ID NO: 9), AAV5 (SEQ ID Nos: 199 and 216 of US20150315612, herein SEQ ID NOs: 105 and 104, respectively), or variants thereof.

[099] In some embodiments, an AAV particle described herein comprises an AAV capsid protein comprising an amino acid sequence provided in WO 2021/230987, e.g., in Table 4 or 6

of WO 2021/230987, the contents of which are hereby incorporated by reference in their entirety.

[0100] In some embodiments the AAV serotype of an AAV particle, e.g., an AAV particle for the vectorized delivery of a GBA1 protein described herein, is AAV9 or AAV5, or a variant of AAV5 or a variant of AAV9. In some embodiments, the AAV particle comprises an AAV5 capsid variant. In some embodiments, the AAV particle comprises an AAV9 capsid variant.

[0101] In some embodiments, the AAV particle, e.g., a recombinant AAV particle described herein, comprises an AAV9 capsid protein. In some embodiments, the AAV9 capsid protein comprises the amino acid sequence of SEQ ID NO: 138. In some embodiments, the nucleic acid sequence encoding the AAV9 capsid protein comprises the nucleotide sequence of SEQ ID NO: 137. In some embodiments, the AAV9 capsid protein comprises an amino acid sequence at least 70% identical to SEQ ID NO: 138, such as, at least 70% identical to, such as, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 138. In some embodiments, the nucleic acid sequence encoding the AAV9 capsid protein comprises a nucleotide sequence at least 70% identical to SEQ ID NO: 137, such as, at least 70% identical to, such as, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to, SEQ ID NO: 137.

[0102] In some embodiments, the AAV particle, e.g., a recombinant AAV particle described herein, comprises an AAV5 capsid protein. In some embodiments, the AAV5 capsid protein comprises the amino acid sequence of SEQ ID NO: 104. In some embodiments, the nucleic acid sequence encoding the AAV5 capsid protein is encoded by the nucleotide sequence of SEQ ID NO: 105. In some embodiments, the AAV5 capsid protein comprises an amino acid sequence at least 70% identical to SEQ ID NO: 104, such as, at least 70% identical to, such as, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to, SEQ ID NO: 104. In some embodiments, the nucleic acid sequence encoding the AAV5 capsid protein is encoded by a nucleotide sequence at least 70% identical to SEQ ID NO: 105, such as, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to, SEQ ID NO: 105.

[0103] In some embodiments, the AAV capsid of an AAV particle, e.g., an AAV particle for the vectorized delivery of a GBA1 protein described herein, allows for blood brain barrier

penetration following intravenous administration. Non-limiting examples of such AAV capsids include AAV9, AAV9 K449R, AAV5, VOY101, VOY201, or AAV capsids comprising a peptide insert such as, but not limited to, AAVPHP.N (PHP.N), AAVPHP.B (PHP.B), PHP.S, G2A3, G2B4, G2B5, G2A12, G2A15, PHP.B2, PHP.B3, AAV2.BR1, or AAVPHP.A (PHP.A).

[0104] In some embodiments, the AAV capsid is an AAV9 comprising an insert comprising the amino acid sequence PLNGAVHLY (SEQ ID NO: 3648), wherein the amino acid sequence of PLNGAVHLY (SEQ ID NO: 3648) is present immediately subsequent to position 586, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid comprises the amino acid sequence of 3636. In some embodiments SEQ ID NO: 3636 comprises the amino acid sequence:

MAADGYLPDWLEDNLSEGIREWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPNGGLDKGEPVNAADA
AALEHDKAYDQQLKAGDNPYLYKNHADAQEFQERLKEDTSFGGNLGRAVFQAKKRLLLEPLGLVEEAAKTAP
GKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTESVPDPQPIGEPPAAPSGVGSGLTMASSGGAP
VADNNEGADGVGSSSGNWHCDSQWLGDVRVITSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTP
WGYFDFNRFHCHFSRDPWQRLINNNWGRPKRLNFKLFNIQVKEVTDNNGVKTIANNLSTVQVFTDSY
QLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENV
PFHSSYAHSQLDRLMNPLIDQYLYLSTKINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVS
TTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVM
TNEEEIKTTNPVATESYGGQVATNHQS **PLNGAVHLY** AQAQTGWVQNQGILPGMVWQDRDVYLQGPWAKIP
HTDGNFHPSPMLGGFGMKHPPQILIKNTFPVADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENS
RWNPEIQYTSNYKSNVNEFAVNTEGVYSEPRPIGTRYLTRNL (SEQ ID NO: 3636).

[0105] In some embodiments, the AAV serotype is selected for use due to its tropism for cells of the central nervous system. In some embodiments, the cells of the central nervous system are neurons. In another embodiment, the cells of the central nervous system are astrocytes.

[0106] In some embodiments, the AAV serotype is selected for use due to its tropism for cells of the muscle(s).

[0107] In some embodiments, the initiation codon for translation of the AAV VP1 capsid protein may be CTG, TTG, or GTG as described in US Patent No. US8163543, the contents of which are herein incorporated by reference in their entirety. In some embodiments, the nucleotide sequence encoding the capsid protein, e.g., a VP1 capsid protein, comprises 3-20 mutations (e.g., substitutions), e.g., 3-15 mutations, 3-10 mutations, 3-5 mutations, 5-20 mutations, 5-15 mutations, 5-10 mutations, 10-20 mutations, 10-15 mutations, 15-20 mutations, 3 mutations, 5 mutations, 10 mutations, 12 mutations, 15 mutations, 18 mutations, or 20 mutations, relative to the nucleotide sequence of SEQ ID NO: 137.

[0108] The present disclosure refers to structural capsid proteins (including VP1, VP2 and VP3) which are encoded by capsid (Cap) genes. These capsid proteins form an outer protein structural shell (*i.e.* capsid) of a viral vector such as AAV. VP capsid proteins synthesized from

Cap polynucleotides generally include a methionine as the first amino acid in the peptide sequence (Met1), which is associated with the start codon (AUG or ATG) in the corresponding Cap nucleotide sequence. However, it is common for a first-methionine (Met1) residue or generally any first amino acid (AA1) to be cleaved off after or during polypeptide synthesis by protein processing enzymes such as Met-aminopeptidases. This “Met/AA-clipping” process often correlates with a corresponding acetylation of the second amino acid in the polypeptide sequence (*e.g.*, alanine, valine, serine, threonine, *etc.*). Met-clipping commonly occurs with VP1 and VP3 capsid proteins but can also occur with VP2 capsid proteins.

[0109] Where the Met/AA-clipping is incomplete, a mixture of one or more (one, two or three) VP capsid proteins comprising the viral capsid may be produced, some of which may include a Met1/AA1 amino acid (Met+/AA+) and some of which may lack a Met1/AA1 amino acid as a result of Met/AA-clipping (Met-/AA-). For further discussion regarding Met/AA-clipping in capsid proteins, see Jin, *et al.* Direct Liquid Chromatography/Mass Spectrometry Analysis for Complete Characterization of Recombinant Adeno-Associated Virus Capsid Proteins. *Hum Gene Ther Methods*. 2017 Oct. 28(5):255-267; Hwang, *et al.* N-Terminal Acetylation of Cellular Proteins Creates Specific Degradation Signals. *Science*. 2010 February 19. 327(5968): 973–977; the contents of which are each incorporated herein by reference in their entirety.

[0110] According to the present disclosure, references to capsid proteins is not limited to either clipped (Met-/AA-) or unclipped (Met+/AA+) and may, in context, refer to independent capsid proteins, viral capsids comprised of a mixture of capsid proteins, and/or polynucleotide sequences (or fragments thereof) which encode, describe, produce or result in capsid proteins of the present disclosure. A direct reference to a “capsid protein” or “capsid polypeptide” (such as VP1, VP2 or VP2) may also comprise VP capsid proteins which include a Met1/AA1 amino acid (Met+/AA+) as well as corresponding VP capsid proteins which lack the Met1/AA1 amino acid as a result of Met/AA-clipping (Met-/AA-).

[0111] Further according to the present disclosure, a reference to a specific “SEQ ID NO:” (whether a protein or nucleic acid) which comprises or encodes, respectively, one or more capsid proteins which include a Met1/AA1 amino acid (Met+/AA+) should be understood to teach the VP capsid proteins which lack the Met1/AA1 amino acid as upon review of the sequence, it is readily apparent any sequence which merely lacks the first listed amino acid (whether or not Met1/AA1).

[0112] As a non-limiting example, reference to a VP1 polypeptide sequence which is 736 amino acids in length and which includes a “Met1” amino acid (Met+) encoded by the

AUG/ATG start codon may also be understood to teach a VP1 polypeptide sequence which is 735 amino acids in length and which does not include the “Met1” amino acid (Met-) of the 736 amino acid Met⁺ sequence. As a second non-limiting example, reference to a VP1 polypeptide sequence which is 736 amino acids in length and which includes an “AA1” amino acid (AA1⁺) encoded by any NNN initiator codon may also be understood to teach a VP1 polypeptide sequence which is 735 amino acids in length and which does not include the “AA1” amino acid (AA1-) of the 736 amino acid AA1⁺ sequence.

[0113] References to viral capsids formed from VP capsid proteins (such as reference to specific AAV capsid serotypes), can incorporate VP capsid proteins which include a Met1/AA1 amino acid (Met⁺/AA1⁺), corresponding VP capsid proteins which lack the Met1/AA1 amino acid as a result of Met/AA1-clipping (Met-/AA1-), and combinations thereof (Met⁺/AA1⁺ and Met-/AA1-).

[0114] As a non-limiting example, an AAV capsid serotype can include VP1 (Met⁺/AA1⁺), VP1 (Met-/AA1-), or a combination of VP1 (Met⁺/AA1⁺) and VP1 (Met-/AA1-). An AAV capsid serotype can also include VP3 (Met⁺/AA1⁺), VP3 (Met-/AA1-), or a combination of VP3 (Met⁺/AA1⁺) and VP3 (Met-/AA1-); and can also include similar optional combinations of VP2 (Met⁺/AA1) and VP2 (Met-/AA1-).

AAV Viral Genome

[0115] In some aspects, the AAV particle of the present disclosure serves as an expression vector comprising a viral genome which encodes a GCase protein. The viral genome can encode a GCase protein and an enhancement, e.g., prosaposin (PSAP) or saposin (Sap) polypeptide or functional variant thereof (e.g., a SapA protein or a SapC protein), a cell penetrating peptide (e.g., an ApoEII peptide, a TAT peptide, or an ApoB peptide), a lysosomal targeting sequence (LTS), or a combination thereof. In some embodiments, expression vectors are not limited to AAV and may be adenovirus, retrovirus, lentivirus, plasmid, vector, or any variant thereof.

[0116] In some embodiments, an AAV particle, e.g., an AAV particle for the vectorized delivery of a GBA1 protein described herein, comprises a viral genome, e.g., an AAV viral genome (e.g., a vector genome or AAV vector genome). In some embodiments, the viral genome, e.g., the AAV viral genome, further comprises an inverted terminal repeat (ITR) region, an enhancer, a promoter, an intron region, a Kozak sequence, an exon region, a nucleic acid encoding a transgene encoding a payload (e.g., a GBA1 protein described herein) with or without an enhancement element, a nucleotide sequence encoding at least one miR binding site (e.g., at least one miR183 binding site), a poly A signal region, or a combination thereof.

Viral Genome Component: Inverted Terminal Repeats (ITRs)

[0117] In some embodiments, the viral genome may comprise at least one inverted terminal repeat (ITR) region. The AAV particles of the present disclosure comprise a viral genome with at least one ITR region and a payload region. In some embodiments, the viral genome has two ITRs. These two ITRs flank the payload region at the 5' and 3' ends. In some embodiments, the ITR functions as an origin of replication comprising a recognition site for replication. In some embodiments, the ITR comprises a sequence region which can be complementary and symmetrically arranged. In some embodiments, the ITR incorporated into a viral genome described herein may be comprised of a naturally occurring polynucleotide sequence or a recombinantly derived polynucleotide sequence.

[0118] The ITRs may be derived from the same serotype as the capsid, selected from any of the serotypes listed in Table 1, or a derivative thereof. The ITR may be of a different serotype than the capsid. In some embodiments, the AAV particle has more than one ITR. In a non-limiting example, the AAV particle has a viral genome comprising two ITRs. In some embodiments, the ITRs are of the same serotype as one another. In another embodiment, the ITRs are of different serotypes. Non-limiting examples include zero, one or both of the ITRs having the same serotype as the capsid. In some embodiments both ITRs of the viral genome of the AAV particle are AAV2 ITRs.

[0119] Independently, each ITR may be about 100 to about 150 nucleotides in length. In some embodiments, the ITR comprises 100-180 nucleotides in length, e.g., about 100-115, about 100-120, about 100-130, about 100-140, about 100-150, about 100-160, about 100-170, about 100-180, about 110-120, about 110-130, about 110-140, about 110-150, about 110-160, about 110-170, about 110-180, about 120-130, about 120-140, about 120-150, about 120-160, about 120-170, about 120-180, about 130-140, about 130-150, about 130-160, about 130-170, about 130-180, about 140-150, about 140-160, about 140-170, about 140-180, about 150-160, about 150-170, about 150-180, about 160-170, about 160-180, or about 170-180 nucleotides in length. In some embodiments, the ITR comprises about 120-140 nucleotides in length, e.g., about 130 nucleotides in length. In some embodiments, the ITRs are 140-142 nucleotides in length, e.g., 141 nucleotides in length. In some embodiments, the ITR comprises 1205-135 nucleotides in length, e.g., 130 nucleotides in length. Non-limiting examples of ITR length are 102, 130, 140, 141, 142, 145 nucleotides in length, and those having at least 95% identity thereto.

[0120] In some embodiments, each ITR is 141 nucleotides in length. In some embodiments, each ITR is 130 nucleotides in length. In some embodiments, the AAV particles comprise two ITRs and one ITR is 141 nucleotides in length and the other ITR is 130 nucleotides in length.

[0121] In some embodiments, the ITR comprises the nucleotide sequence of any one of SEQ ID NOs: 1829, 1830, or 1862, or a nucleotide sequence substantially identical (e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical, or 100% identical to) to any of the aforesaid sequences. In some embodiments, the ITR comprises the nucleotide sequence of any of SEQ ID NOs: 1860, 1861, 1863, or 1864, or a nucleotide sequence having one, two, or three but no more than four modifications, e.g., substitutions, relative to SEQ ID NOs: 1860, 1861, 1863, or 1864.

Viral Genome Component: Promoters and Expression Enhancers

[0122] In some embodiments, the payload region of the viral genome comprises at least one element to enhance the transgene target specificity and expression. See, e.g., Powell *et al.* Viral Expression Cassette Elements to Enhance Transgene Target Specificity and Expression in Gene Therapy, 2015; the contents of which are herein incorporated by reference in their entirety. Non-limiting examples of elements to enhance the transgene target specificity and expression include promoters, endogenous miRNAs, post-transcriptional regulatory elements (PREs), polyadenylation (PolyA) signal sequences, upstream enhancers (USEs), CMV enhancers, and introns.

[0123] In some embodiments, expression of the polypeptides in a target cell may be driven by a specific promoter, including but not limited to, a promoter that is species specific, inducible, tissue-specific, or cell cycle-specific (Parr *et al.*, *Nat. Med.* 3:1145-9 (1997); the contents of which are herein incorporated by reference in their entirety).

[0124] In some embodiments, the viral genome provides expression of a GBA1 protein in a target tissue (e.g., the CNS). In some embodiments, the promoter is deemed to be efficient when it drives expression of the polypeptide(s) encoded in the payload region of the viral genome of the AAV particle.

[0125] In some embodiments, the promoter is a promoter deemed to be efficient when it drives expression in the cell or tissue being targeted (e.g., the CNS).

[0126] In some embodiments, the promoter drives expression of the GCase, GCase and SapA, or GCase and SapC protein(s) for a period of time in targeted tissues. Expression driven by a promoter may be for a period of 1 hour, 2, hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 3 weeks, 22 days, 23 days, 24 days, 25 days, 26 days,

27 days, 28 days, 29 days, 30 days, 31 days, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years or more than 10 years. Expression may be for 1-5 hours, 1-12 hours, 1-2 days, 1-5 days, 1-2 weeks, 1-3 weeks, 1-4 weeks, 1-2 months, 1-4 months, 1-6 months, 2-6 months, 3-6 months, 3-9 months, 4-8 months, 6-12 months, 1-2 years, 1-5 years, 2-5 years, 3-6 years, 3-8 years, 4-8 years, or 5-10 years.

[0127] In some embodiments, the promoter drives expression of a polypeptide (e.g., a GCase polypeptide, a GCase polypeptide and a prosaposin (PSAP) polypeptide, a GCase polypeptide and a SapA polypeptide, a GCase polypeptide and a SapC polypeptide, a GCase polypeptide and a cell penetrating peptide (e.g., an ApoEII peptide, a TAT peptide, and/or a ApoB peptide), or a GCase polypeptide and a lysosomal targeting peptide) for at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, 13 years, 14 years, 15 years, 16 years, 17 years, 18 years, 19 years, 20 years, 21 years, 22 years, 23 years, 24 years, 25 years, 26 years, 27 years, 28 years, 29 years, 30 years, 31 years, 32 years, 33 years, 34 years, 35 years, 36 years, 37 years, 38 years, 39 years, 40 years, 41 years, 42 years, 43 years, 44 years, 45 years, 46 years, 47 years, 48 years, 49 years, 50 years, 55 years, 60 years, 65 years, or more than 65 years.

[0128] Promoters may be naturally occurring or non-naturally occurring. Non-limiting examples of promoters include viral promoters, plant promoters and mammalian promoters. In some embodiments, the promoters may be human promoters. In some embodiments, the promoter may be truncated.

[0129] In some embodiments, the viral genome comprises a promoter that results in expression in one or more, e.g., multiple, cells and/or tissues, e.g., a ubiquitous promoter. In some embodiments, a promoter which drives or promotes expression in most mammalian tissues includes, but is not limited to, human elongation factor 1 α -subunit (EF1 α), cytomegalovirus (CMV) immediate-early enhancer and/or promoter, chicken β -actin (CBA) and its derivative CAG, β glucuronidase (GUSB), and ubiquitin C (UBC). Tissue-specific expression elements can be used to restrict expression to certain cell types such as, but not limited to, CNS-specific promoters, B cell promoters, monocyte promoters, leukocyte promoters, macrophage promoters, pancreatic acinar cell promoters, endothelial cell promoters, lung tissue promoters, astrocyte

promoters, or various specific nervous system cell- or tissue-type promoters which can be used to restrict expression to neurons, astrocytes, or oligodendrocytes, for example.

[0130] In some embodiments, the viral genome comprises a nervous system specific promoter, e.g., a promoter that results in expression of a payload in a neuron, an astrocyte, and/or an oligodendrocyte. Non-limiting examples of tissue-specific expression elements for neurons include neuron-specific enolase (NSE), platelet-derived growth factor (PDGF), platelet-derived growth factor B-chain (PDGF- β), synapsin (Syn), synapsin 1 (Syn1), methyl-CpG binding protein 2 (MeCP2), Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), metabotropic glutamate receptor 2 (mGluR2), neurofilament light (NFL) or heavy (NFH), β -globin minigene β 2, preproenkephalin (PPE), enkephalin (Enk) and excitatory amino acid transporter 2 (EAAT2) promoters. Non-limiting examples of tissue-specific expression elements for astrocytes include glial fibrillary acidic protein (GFAP) and EAAT2 promoters. A non-limiting example of a tissue-specific expression element for oligodendrocytes includes the myelin basic protein (MBP) promoter. Prion promoter represents an additional tissue specific promoter useful for driving protein expression in CNS tissue (see Loftus, Stacie K., *et al.* Human molecular genetics 11.24 (2002): 3107-3114, the disclosure of which is incorporated by reference in its entirety).

[0131] In some embodiments, the promoter may be less than 1 kb. The promoter may have a length of 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, or more than 800 nucleotides. The promoter may have a length of 200-300, 200-400, 200-500, 200-600, 200-700, 200-800, 300-400, 300-500, 300-600, 300-700, 300-800, 400-500, 400-600, 400-700, 400-800, 500-600, 500-700, 500-800, 600-700, 600-800, or 700-800 nucleotides.

[0132] In some embodiments, the promoter may be a combination of two or more components of the same or different starting or parental promoters such as, but not limited to, CMV and CBA. Each component may have a length of 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, or more than 800 nucleotides. Each component may have a length of 200-300, 200-400, 200-500, 200-600, 200-700, 200-800, 300-400, 300-500, 300-600, 300-700, 300-800, 400-500, 400-600, 400-700, 400-800, 500-600, 500-700, 500-800, 600-700, 600-800

or 700-800 nucleotides. In some embodiments, the promoter is a combination of a 382 nucleotide CMV-enhancer sequence and a 260 nucleotide CBA-promoter sequence. In some embodiments, the promoter is a combination of a 380 nucleotide CMV-enhancer sequence (e.g., comprising the nucleotide sequence of SEQ ID NO: 1831) and a 260 nucleotide CBA-promoter sequence (e.g., comprising the nucleotide sequence of SEQ ID NO: 1834).

[0133] In some embodiments, the viral genome comprises a ubiquitous promoter. Non-limiting examples of ubiquitous promoters include CMV, CBA (including derivatives CAG, CB6, CBh, *etc.*), EF-1 α , PGK, UBC, GUSB (hGBp), and UCOE (promoter of HNRPA2B1-CBX3). In some embodiments, the viral genome comprises an EF-1 α promoter or EF-1 α promoter variant.

[0134] In some embodiments, the promoter is a ubiquitous promoter as described in Yu et al. (Molecular Pain 2011, 7:63), Soderblom et al. (E. Neuro 2015), Gill et al., (Gene Therapy 2001, Vol. 8, 1539-1546), and Husain et al. (Gene Therapy 2009), each of which are incorporated by reference in their entirety.

[0135] In some embodiments, the promoter is not cell specific.

[0136] In some embodiments, the promoter is a ubiquitin c (UBC) promoter. The UBC promoter may have a size of 300-350 nucleotides. As a non-limiting example, the UBC promoter is 332 nucleotides. In some embodiments, the promoter is a β -glucuronidase (GUSB) promoter. The GUSB promoter may have a size of 350-400 nucleotides. As a non-limiting example, the GUSB promoter is 378 nucleotides. In some embodiments, the promoter is a neurofilament light (NFL) promoter. The NFL promoter may have a size of 600-700 nucleotides. As a non-limiting example, the NFL promoter is 650 nucleotides. In some embodiments, the promoter is a neurofilament heavy (NFH) promoter. The NFH promoter may have a size of 900-950 nucleotides. As a non-limiting example, the NFH promoter is 920 nucleotides. In some embodiments, the promoter is a scn8a promoter. The scn8a promoter may have a size of 450-500 nucleotides. As a non-limiting example, the scn8a promoter is 470 nucleotides.

[0137] In some embodiments, the promoter is a phosphoglycerate kinase 1 (PGK) promoter.

[0138] In some embodiments, the promoter is a chicken β -actin (CBA) promoter, or a functional variant thereof.

[0139] In some embodiments, the promoter is a CB6 promoter, or a functional variant thereof.

[0140] In some embodiments, the promoter is a CB promoter, or a functional variant thereof. In some embodiments, the promoter is a minimal CB promoter, or a functional variant thereof.

[0141] In some embodiments, the promoter is a CBA promoter, or functional variant thereof. In some embodiments, the promoter is a minimal CBA promoter, or functional variant thereof.

[0142] In some embodiments, the promoter is a cytomegalovirus (CMV) promoter, or a functional variant thereof.

[0143] In some embodiments, the promoter is a CAG promoter, or a functional variant thereof.

[0144] In some embodiments, the promoter is an EF1 α promoter or functional variant thereof.

[0145] In some embodiments, the promoter is a GFAP promoter (as described, for example, in Zhang, Min, *et al.* Journal of neuroscience research 86.13 (2008): 2848-2856, the disclosure of which is incorporated by reference in its entirety) to drive expression of a GCase polypeptide, or a GCase polypeptide and an enhancement element (e.g., GCase and SapA, or GCase and SapC protein expression) in astrocytes.

[0146] In some embodiments, the promoter is a synapsin promoter, or a functional variant thereof.

[0147] In some embodiments, the promoter is an RNA pol III promoter. As a non-limiting example, the RNA pol III promoter is U6. As a non-limiting example, the RNA pol III promoter is H1.

[0148] In some embodiments, the viral genome comprises two promoters. As a non-limiting example, the promoters are an EF1 α promoter and a CMV promoter.

[0149] In some embodiments, the viral genome comprises an enhancer element, a promoter and/or a 5'UTR intron. The enhancer element, also referred to herein as an "enhancer," may be, but is not limited to, a CMV enhancer, the promoter may be, but is not limited to, a CMV, CBA, UBC, GUSB, NSE, Synapsin, MeCP2, and GFAP promoter and the 5'UTR/intron may be, but is not limited to, SV40, and CBA-MVM. As a non-limiting example, the enhancer, promoter and/or intron used in combination may be: (1) CMV enhancer, CMV promoter, SV40 5'UTR intron; (2) CMV enhancer, CBA promoter, SV 40 5'UTR intron; (3) CMV enhancer, CBA promoter, CBA-MVM 5'UTR intron; (4) UBC promoter; (5) GUSB promoter; (6) NSE promoter; (7) Synapsin promoter; (8) MeCP2 promoter; and (9) GFAP promoter.

[0150] In some embodiments, the viral genome comprises an enhancer. In some embodiments, the enhancer comprises a CMVie enhancer.

[0151] In some embodiments the viral genome comprises a CMVie enhancer and a CB promoter. In some embodiments, the viral genome comprises a CMVie enhancer and a CMV promoter (e.g., a CMV promoter region). In some embodiments, the viral genome comprises a

CMVie enhancer, a CBA promoter or functional variant thereof, and an intron (e.g., a CAG promoter).

[0152] In some embodiments, the viral genome comprises an engineered promoter. In another embodiment, the viral genome comprises a promoter from a naturally expressed protein.

[0153] In some embodiments, a CBA promoter is used in a viral genome of an AAV particle described herein, e.g., a viral genome encoding a GCaP protein, or a GCaP protein and an enhancement element (e.g., a GCaP and SapA proteins, GCaP and SapC proteins, or GCaP protein and a cell penetrating peptide or variants thereof). In some embodiments, the CBA promoter is engineered for optimal expression of a GCaP polypeptide or a GCaP polypeptide and an enhancement element described herein (e.g., a prosaposin or saposin protein or variant thereof; a cell penetrating peptide or variant thereof; or a lysosomal targeting signal).

Viral Genome Component: Introns

[0154] In some embodiments, the vector genome comprises at least one intron or a fragment or derivative thereof. In some embodiments, the at least one intron may enhance expression of a GCaP protein and/or an enhancement element described herein (e.g., a prosaposin protein or a SapC protein or variant thereof; a cell penetrating peptide (e.g., a ApoEII peptide, a TAT peptide, or a ApoB peptide) or variant thereof; and/or a lysosomal targeting signal) (see *e.g.*, Powell *et al.* Viral Expression Cassette Elements to Enhance Transgene Target Specificity and Expression in Gene Therapy, 2015; the contents of which are herein incorporated by reference in their entirety). Non-limiting examples of introns include, MVM (67-97 bps), F.IX truncated intron 1 (300 bps), β -globin SD/immunoglobulin heavy chain splice acceptor (250 bps), adenovirus splice donor/immunoglobulin splice acceptor (500 bps), SV40 late splice donor/splice acceptor (19S/16S) (180 bps), and hybrid adenovirus splice donor/IgG splice acceptor (230 bps).

[0155] In some embodiments, the intron may be 100-500 nucleotides in length. The intron may have a length of 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490 or 500 nucleotides. The intron may have a length between 80-100, 80-120, 80-140, 80-160, 80-180, 80-200, 80-250, 80-300, 80-350, 80-400, 80-450, 80-500, 200-300, 200-400, 200-500, 300-400, 300-500, or 400-500 nucleotides.

[0156] In some embodiments, the intron may be 100-600 nucleotides in length. In some embodiments, the intron is 566 nucleotides in length.

[0157] In some embodiments, the AAV vector may comprise an SV40 intron or fragment or

variant thereof. In some embodiments, the promoter may be a CMV promoter. In some embodiments, the promoter may be CBA. In some embodiments, the promoter may be H1.

[0158] In some embodiments, the AAV vector may comprise a beta-globin intron or a fragment or variant thereof. In some embodiments, the intron comprises one or more human beta-globin sequences (e.g., including fragments/variants thereof). In some embodiments the promoter may be a CB promoter. In some embodiments, the promoter comprises a CMV promoter. In some embodiments, the promoter comprises a minimal CBA promoter.

[0159] In some embodiments, the encoded protein(s) may be located downstream of an intron in an expression vector such as, but not limited to, SV40 intron or beta globin intron or others known in the art. Further, the encoded GBA1 protein may also be located upstream of the polyadenylation sequence in an expression vector. In some embodiments, the encoded proteins may be located within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more than 30 nucleotides downstream from the promoter comprising an intron (e.g., 3' relative to the promoter comprising an intron) and/or upstream of the polyadenylation sequence (e.g., 5' relative to the polyadenylation sequence) in an expression vector. In some embodiments, the encoded GBA1 protein may be located within 1-5, 1-10, 1-15, 1-20, 1-25, 1-30, 5-10, 5-15, 5-20, 5-25, 5-30, 10-15, 10-20, 10-25, 10-30, 15-20, 15-25, 15-30, 20-25, 20-30, or 25-30 nucleotides downstream from the intron (e.g., 3' relative to the intron) and/or upstream of the polyadenylation sequence (e.g., 5' relative to the polyadenylation sequence) in an expression vector. In some embodiments, the encoded proteins may be located within the first 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, or more than 25% of the nucleotides downstream from the intron (e.g., 3' relative to the intron) and/or upstream of the polyadenylation sequence (e.g., 5' relative to the polyadenylation sequence) in an expression vector. In some embodiments, the encoded proteins may be located within the first 1-5%, 1-10%, 1-15%, 1-20%, 1-25%, 5-10%, 5-15%, 5-20%, 5-25%, 10-15%, 10-20%, 10-25%, 15-20%, 15-25%, or 20-25% of the sequence downstream from the intron (e.g., 3' relative to the intron) and/or upstream of the polyadenylation sequence (e.g., 5' relative to the polyadenylation sequence) in an expression vector.

[0160] In certain embodiments, the intron sequence is not an enhancer sequence. In some embodiments, the intron sequence is not a sub-component of a promoter sequence. In some embodiments, the intron sequence is a sub-component of a promoter sequence.

Viral Genome Component: Untranslated Regions (UTRs)

[0161] In some embodiments, a wild type untranslated region (UTR) of a gene is transcribed but not translated. Generally, the 5' UTR starts at the transcription start site and ends at the start

codon and the 3' UTR starts immediately following the stop codon and continues until the termination signal for transcription.

[0162] Features typically found in abundantly expressed genes of specific target organs may be engineered into UTRs to enhance the stability and protein production. As a non-limiting example, a 5' UTR from mRNA normally expressed in the liver (*e.g.*, albumin, serum amyloid A, Apolipoprotein A/B/E, transferrin, alpha fetoprotein, erythropoietin, or Factor VIII) may be used in the viral genomes of the AAV particles of the disclosure to enhance expression in hepatic cell lines or liver.

[0163] In some embodiments, the viral genome encoding a transgene described herein (*e.g.*, a transgene encoding a GBA1 protein) comprises a Kozak sequence. While not wishing to be bound by theory, wild-type 5' untranslated regions (UTRs) include features that play roles in translation initiation. Kozak sequences, which are commonly known to be involved in the process by which the ribosome initiates translation of many genes, are usually included in 5' UTRs. Kozak sequences have the consensus CCR(A/G)CCAUGG, where R is a purine (adenine or guanine) three bases upstream of the start codon (ATG), which is followed by another 'G'.

[0164] In some embodiments, the 5' UTR in the viral genome includes a Kozak sequence.

[0165] In some embodiments, the 5' UTR in the viral genome does not include a Kozak sequence.

[0166] While not wishing to be bound by theory, wild-type 3' UTRs are known to have stretches of adenosines and uridines embedded therein. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995, the contents of which are herein incorporated by reference in their entirety): Class I AREs, such as, but not limited to, c-Myc and MyoD, contain several dispersed copies of an AUUUA motif within U-rich regions. Class II AREs, such as, but not limited to, GM-CSF and TNF- α , possess two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Class III AREs, such as, but not limited to, c-Jun and Myogenin, are less well defined. These U rich regions do not contain an AUUUA motif. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3' UTR of nucleic acid molecules will lead to HuR binding and thus, stabilization of the message *in vivo*.

[0167] Introduction, removal or modification of 3' UTR AU rich elements (AREs) can be used to modulate the stability of polynucleotides. When engineering specific polynucleotides,

e.g., payload regions of viral genomes, one or more copies of an ARE can be introduced to make polynucleotides less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein.

[0168] In some embodiments, the 3' UTR of the viral genome may include an oligo(dT) sequence for templated addition of a poly-A tail.

[0169] Any UTR from any gene known in the art may be incorporated into the viral genome of the AAV particle. These UTRs, or portions thereof, may be placed in the same orientation as in the gene from which they were selected or they may be altered in orientation or location. In some embodiments, the UTR used in the viral genome of the AAV particle may be inverted, shortened, lengthened, or made with one or more other 5' UTRs or 3' UTRs known in the art. As used herein, the term "altered," as it relates to a UTR, means that the UTR has been changed in some way in relation to a reference sequence. For example, a 3' or 5' UTR may be altered relative to a wild type or native UTR by the change in orientation or location as taught above or may be altered by the inclusion of additional nucleotides, deletion of nucleotides, swapping or transposition of nucleotides.

[0170] In some embodiments, the viral genome of the AAV particle comprises at least one artificial UTR, which is not a variant of a wild type UTR.

[0171] In some embodiments, the viral genome of the AAV particle comprises UTRs which have been selected from a family of transcripts whose proteins share a common function, structure, feature, or property.

Viral Genome Component: miR Binding Site

[0172] Tissue- or cell-specific expression of the AAV viral particles of the invention can be enhanced by introducing tissue- or cell-specific regulatory sequences, *e.g.*, promoters, enhancers, microRNA binding sites, *e.g.*, a detargeting site. Without wishing to be bound by theory, it is believed that an encoded miR binding site can modulate, *e.g.*, prevent, suppress, or otherwise inhibit, the expression of a gene of interest on the viral genome of the invention, based on the expression of the corresponding endogenous microRNA (miRNA) or a corresponding controlled exogenous miRNA in a tissue or cell, *e.g.*, a non-targeting cell or tissue. In some embodiments, a miR binding site modulates, *e.g.*, reduces, expression of the payload encoded by a viral genome of an AAV particle described herein in a cell or tissue where the corresponding mRNA is expressed. In some embodiments, the miR binding site modulates, *e.g.*, reduces,

expression of the encoded GBA1 protein in a cell or tissue of the DRG, liver, hematopoietic lineage, or a combination thereof.

[0173] In some embodiments, the viral genome of an AAV particle described herein comprises a nucleotide sequence encoding a microRNA binding site, *e.g.*, a detargeting site. In some embodiments, the viral genome of an AAV particle described herein comprises a nucleotide sequence encoding a miR binding site, a microRNA binding site series (miR BSs), or a reverse complement thereof.

[0174] In some embodiments, the nucleotide sequence encoding the miR binding site series or the miR binding site is located in the 3'-UTR region of the viral genome (*e.g.*, 3' relative to the nucleic acid sequence encoding a payload), *e.g.*, before the polyA sequence, 5'-UTR region of the viral genome (*e.g.*, 5' relative to the nucleic acid sequence encoding a payload), or both.

[0175] In some embodiments, the encoded miR binding site series comprise at least 1-5 copies, *e.g.*, 1-3, 2-4, or 3-5 copies, or at least 1, at least 2, at least 3, at least 4, at least 5 or more copies of a miR binding site (miR BS). In some embodiments, the encoded miR binding site series comprises 4 copies of a miR binding site. In some embodiments, all copies are identical, *e.g.*, comprise the same miR binding site. In some embodiments, the miR binding sites within the encoded miR binding site series are continuous and not separated by a spacer. In some embodiments, the miR binding sites within an encoded miR binding site series are separated by a spacer, *e.g.*, a non-coding sequence. In some embodiments, the spacer is about 1 to about 6 nucleotides or about 5 to about 10 nucleotides, *e.g.*, about 7-8 nucleotides, nucleotides in length. In some embodiments, the spacer is about 8 nucleotides in length. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848.

[0176] In some embodiments, the encoded miR binding site series comprise at least 1-5 copies, *e.g.*, 1-3, 2-4, or 3-5 copies, or at least 1, at least 2, at least 3, at least 4, at least 5 or more copies of a miR binding site (miR BS). In some embodiments, at least 1, at least 2, at least 3, at least 4, at least 5, or all of the copies are different, *e.g.*, comprise a different miR binding site. In some embodiments, the miR binding sites within the encoded miR binding site series are continuous and not separated by a spacer. In some embodiments, the miR binding sites within an encoded miR binding site series are separated by a spacer, *e.g.*, a non-coding sequence. In some embodiments, the spacer is about 1 to about 6 nucleotides or about 5 to about 10 nucleotides, *e.g.*, about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments,

the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA (SEQ ID NO: 1848), or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of GATAGTTA (SEQ ID NO: 1848).

[0177] In some embodiments, the encoded miR binding site is substantially identical (*e.g.*, at least 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% identical), to the miR in the host cell. In some embodiments, the encoded miR binding site comprises at least 1, 2, 3, 4, or 5 mismatches or no more than 6, 7, 8, 9, or 10 mismatches to a miR in the host cell. In some embodiments, the mismatched nucleotides are contiguous. In some embodiments, the mismatched nucleotides are non-contiguous. In some embodiments, the mismatched nucleotides occur outside the seed region-binding sequence of the miR binding site, such as at one or both ends of the miR binding site. In some embodiments, the encoded miR binding site is 100% identical to the miR in the host cell.

[0178] In some embodiments, the nucleotide sequence encoding the miR binding site is substantially complementary (*e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or 100% complementary), to the miR in the host cell. In some embodiments, the sequence complementary to the nucleotide sequence encoding the miR binding site comprises at least 1, at least 2, at least 3, at least 4, or at least 5 mismatches or no more than 6, no more than 7, no more than 8, no more than 9, or no more than 10 mismatches relative to the corresponding miR in the host cell. In some embodiments, the mismatched nucleotides are contiguous. In some embodiments, the mismatched nucleotides are non-contiguous. In some embodiments, the mismatched nucleotides occur outside the seed region-binding sequence of the miR binding site, such as at one or both ends of the miR binding site. In some embodiments, the encoded miR binding site is 100% complementary to the miR in the host cell.

[0179] In some embodiments, the encoded miR binding site or the encoded miR binding site series is about 10 to about 125 nucleotides in length, *e.g.*, about 10 to about 50 nucleotides, about 10 to about 100 nucleotides, about 50 to about 100 nucleotides, about 50 to about 125 nucleotides, or about 100 to about 125 nucleotides in length. In some embodiments, an encoded miR binding site or the encoded miR binding site series is about 7 to about 28 nucleotides in length, *e.g.*, about 8-28 nucleotides, about 7-28 nucleotides, about 8-18 nucleotides, about 12-28 nucleotides, about 20-26 nucleotides, about 22 nucleotides, about 24 nucleotides, or about 26 nucleotides in length, and optionally comprises at least one consecutive region (*e.g.*, 7 or 8

nucleotides) complementary (e.g., full complementary or partially complementary) to the seed sequence of a miRNA (*e.g.*, a miR122, a miR142, a miR183).

[0180] In some embodiments, the encoded miR binding site or the encoded miR binding site series is 22 nucleotides in length.

[0181] In some embodiments, the encoded miR binding site is complementary (e.g., fully complementary or partially complementary) to a miR expressed in liver or hepatocytes, such as miR122. In some embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR122 binding site sequence. In some embodiments, the encoded miR122 binding site comprises the nucleotide sequence of ACAAACACCATTGTCACACTCCA (SEQ ID NO: 1865), or a nucleotide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least at least 95%, at least 99%, or 100% sequence identity, or having at least one, at least two, at least three, at least four, at least five, at least six, or at least seven modifications but no more than ten modifications to SEQ ID NO: 1865, *e.g.*, wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 3, 4, or 5 copies of the encoded miR122 binding site, *e.g.*, an encoded miR122 binding site series, optionally wherein the encoded miR122 binding site series comprises the nucleotide sequence of:

ACAAACACCATTGTCACACTCCACACAAACACCATTGTCACACTCCACACAAACACCATTGTCACTCCA (SEQ ID NO: 1866), or a nucleotide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications but no more than ten modifications to SEQ ID NO: 1866, *e.g.*, wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, at least two of the encoded miR122 binding sites are connected directly, *e.g.*, without a spacer. In other embodiments, at least two of the encoded miR122 binding sites are separated by a spacer, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length, which is located between two or more consecutive encoded miR122 binding site sequences. In embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, *e.g.*, about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848.

[0182] In some embodiments, the encoded miR binding site is complementary (e.g., fully complementary or partially complementary) to a miR expressed in hematopoietic lineage, including immune cells (e.g., antigen presenting cells or APC, including dendritic cells (DCs), macrophages, and B-lymphocytes). In some embodiments, the encoded miR binding site is complementary (e.g., fully complementary or partially complementary) to a miR expressed in hematopoietic lineage comprises a nucleotide sequence disclosed, e.g., in US 2018/0066279, the contents of which are incorporated by reference herein in its entirety.

[0183] In some embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR-142-3p binding site sequence. In some embodiments, the encoded miR-142-3p binding site comprises the nucleotide sequence of TCCATAAAGTAGGAAACACTACA (SEQ ID NO: 1869), a nucleotide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, at least two, at least three, at least four, at least five, at least six, or at least seven modifications but no more than ten modifications to SEQ ID NO: 1842, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 3, at least 4, or at least 5 copies of an encoded miR-142-3p binding site, e.g., an encoded miR-142-3p binding site series. In some embodiments, the at least 3, at least 4, or at least 5 copies (e.g., 4 copies) of the encoded miR-142-3p binding site are continuous (e.g., not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to about 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848.

[0184] In some embodiments, the encoded miR binding site is complementary (e.g., fully complementary or partially complementary) to a miR expressed in a DRG (dorsal root ganglion) neuron, e.g., a miR183, a miR182, and/or miR96 binding site. In some embodiments, the encoded miR binding site is complementary (e.g., fully complementary or partially complementary) to a miR expressed in expressed in a DRG neuron. In some embodiments, the encoded miR binding site comprises a nucleotide sequence disclosed, e.g., in WO2020/132455, the contents of which are incorporated by reference herein in its entirety.

[0185] In some embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR183 binding site sequence. In some embodiments, the encoded miR183 binding site comprises the nucleotide sequence of AGTGAATTCTACCAGTGCCATA (SEQ ID NO: 1847), or a nucleotide sequence at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, at least two, at least three, at least four, at least five, at least six, or at least seven modifications but no more than ten modifications to SEQ ID NO: 1847, *e.g.*, wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the sequence complementary (*e.g.*, fully complementary or partially complementary) to the seed sequence corresponds to the double underlined of the encoded miR-183 binding site sequence. In some embodiments, the viral genome comprises at least comprises at least 3, at least 4, or at least 5 copies (*e.g.*, 4 copies) of the encoded miR183 binding site, *e.g.* an encoded miR183 binding site. In some embodiments, the viral genome comprises at least comprises 4 copies of the encoded miR183 binding site. In some embodiments, the viral genome comprises an encoded miR183 binding site comprising 4 copies of a miR183 binding site. In some embodiments, the at least 3, at least 4, or at least 5 copies (*e.g.*, 4 copies) of the encoded miR183 binding site are continuous (*e.g.*, not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to about 6 nucleotides or about 5 to about 10 nucleotides, *e.g.*, about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer comprises the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, at least two, or at least three modifications, but no more than four modifications of SEQ ID NO: 1848. In some embodiments, the encoded miR183 binding site series comprises the nucleotide sequence of SEQ ID NO: 1849, or a nucleotide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, at least two, at least three, at least four, at least five, at least six, or at least seven modifications but no more than ten modifications to SEQ ID NO: 1849.

[0186] In some embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR182 binding site sequence. In some embodiments, the encoded miR182 binding site comprises, the nucleotide sequence of AGTGTGAGTTCTACCATTGCCAAA (SEQ ID NO: 1867), a nucleotide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, at least two, at least three, at least four, at least

five, at least six, or at least seven modifications but no more than ten modifications to SEQ ID NO: 1867, *e.g.*, wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 3, at least 4, or at least 5 copies of the encoded miR182 binding site, *e.g.*, an encoded miR182 binding site series. In some embodiments, the at least 3, at least 4, or at least 5 copies (*e.g.*, 4 copies) of the encoded miR182 binding site are continuous (*e.g.*, not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to about 6 nucleotides or about 5 to about 10 nucleotides, *e.g.*, about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer comprises the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, at least two, or at least three modifications, but no more than four modifications of SEQ ID NO: 1848.

[0187] In some embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR96 binding site sequence. In some embodiments, the encoded miR96 binding site comprises the nucleotide sequence of AGCAAAAATGTGCTAGTGCCAAA (SEQ ID NO: 1868), a sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, at least two, at least three, at least four, at least five, at least six, or at least seven modifications but no more than ten modifications to SEQ ID NO: 1868, *e.g.*, wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 3, at least 4, or at least 5 copies of the encoded miR96 binding site, *e.g.*, an encoded miR96 binding site series. In some embodiments, the at least 3, at least 4, or at least 5 copies (*e.g.*, 4 copies) of the encoded miR96 binding site are continuous (*e.g.*, not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to about 6 nucleotides or about 5 to about 10 nucleotides, *e.g.*, about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer comprises the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, at least two, or at least three modifications, but no more than four modifications of SEQ ID NO: 1848.

[0188] In some embodiments, the encoded miR binding site series comprises a miR122 binding site, a miR142 binding site, a miR183 binding site, a miR182 binding site, a miR 96 binding site, or a combination thereof. In some embodiments, the encoded miR binding site series comprises at least 3, at least 4, or at least 5 copies of a miR122 binding site, a miR142 binding site, a miR183 binding site, a miR182 binding site, a miR 96 binding site, or a combination thereof. In some embodiments, at least two of the encoded miR binding sites are

connected directly, *e.g.*, without a spacer. In other embodiments, at least two of the encoded miR binding sites are separated by a spacer, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length, which is located between two or more consecutive encoded miR binding site sequences. In embodiments, the spacer is at least about 5 to about 10 nucleotides, *e.g.*, about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848.

[0189] In some embodiments, an encoded miR binding site series comprises at least 3-5 copies (*e.g.*, 4 copies) of a combination of at least two, three, four, five, or all of a miR122 binding site, a miR142 binding site, a miR183 binding site, a miR182 binding site, a miR96 binding site, wherein each of the miR binding sites within the series are continuous (*e.g.*, not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to about 6 nucleotides or about 5 to about 10 nucleotides, *e.g.*, about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of SEQ ID NO: 1848, or a nucleotide sequence having at least one, two, or three modifications, but no more than four modifications of SEQ ID NO: 1848.

Viral Genome Component: Polyadenylation Sequence

[0190] In some embodiments, the viral genome of the AAV particles of the present disclosure comprises at least one polyadenylation (polyA) sequence. The viral genome of the AAV particle may comprise a polyadenylation sequence between the 3' end of the payload coding sequence and the 5' end of the 3'UTR. In some embodiments, the polyA signal region is positioned 3' relative to the nucleic acid comprising the transgene encoding the payload, *e.g.*, a GBA1 protein described herein.

[0191] In some embodiments, the polyA signal region comprises a length of about 100-600 nucleotides, *e.g.*, about 100-500 nucleotides, about 100-400 nucleotides, about 100-300 nucleotides, about 100-200 nucleotides, about 200-600 nucleotides, about 200-500 nucleotides, about 200-400 nucleotides, about 200-300 nucleotides, about 300-600 nucleotides, about 300-500 nucleotides, about 300-400 nucleotides, about 400-600 nucleotides, about 400-500 nucleotides, or about 500-600 nucleotides. In some embodiments, the polyA signal region comprises a length of about 100 to about 150 nucleotides, *e.g.*, about 127 nucleotides. In some

embodiments, the polyA signal region comprises a length of about 450 to about 500 nucleotides, e.g., about 477 nucleotides. In some embodiments, the polyA signal region comprises a length of about 520 to about 560 nucleotides, e.g., about 552 nucleotides. In some embodiments, the polyA signal region comprises a length of about 127 nucleotides.

[0192] In some embodiments, the viral genome comprises a human growth hormone (hGH) polyA sequence. In some embodiments, the viral genome comprises an hGH polyA as described above and a payload region encoding the GCase protein, or the GCase and an enhancement element (e.g., a prosaposin, SapA, or SapC protein, or variant thereof; a cell penetrating peptide (e.g., an ApoEII peptide, a TAT peptide, or an ApoB peptide); or a lysosomal targeting peptide) e.g., encoding a sequence as provided in Tables 3 and 4 or fragment or variant thereof.

Viral Genome Component: Filler Sequence

[0193] In some embodiments, the viral genome comprises one or more filler sequences. The filler sequence may be a wild-type sequence or an engineered sequence. A filler sequence may be a variant of a wild-type sequence. In some embodiments, a filler sequence is a derivative of human albumin.

[0194] In some embodiments, the viral genome comprises one or more filler sequences in order to have the length of the viral genome be the optimal size for packaging. In some embodiments, the viral genome comprises at least one filler sequence in order to have the length of the viral genome be about 2.3 kb. In some embodiments, the viral genome comprises at least one filler sequence in order to have the length of the viral genome be about 4.6 kb.

[0195] In some embodiments, the viral genome is a single stranded (ss) viral genome and comprises one or more filler sequences that, independently or together, have a length about between 0.1 kb - 3.8 kb, such as, but not limited to, 0.1 kb, 0.2 kb, 0.3 kb, 0.4 kb, 0.5 kb, 0.6 kb, 0.7 kb, 0.8 kb, 0.9 kb, 1 kb, 1.1 kb, 1.2 kb, 1.3 kb, 1.4 kb, 1.5 kb, 1.6 kb, 1.7 kb, 1.8 kb, 1.9 kb, 2 kb, 2.1 kb, 2.2 kb, 2.3 kb, 2.4 kb, 2.5 kb, 2.6 kb, 2.7 kb, 2.8 kb, 2.9 kb, 3 kb, 3.1 kb, 3.2 kb, 3.3 kb, 3.4 kb, 3.5 kb, 3.6 kb, 3.7 kb, or 3.8 kb. In some embodiments, the total length filler sequence in the vector genome is 3.1 kb. In some embodiments, the total length filler sequence in the vector genome is 2.7 kb. In some embodiments, the total length filler sequence in the vector genome is 0.8 kb. In some embodiments, the total length filler sequence in the vector genome is 0.4 kb. In some embodiments, the length of each filler sequence in the vector genome is 0.8 kb. In some embodiments, the length of each filler sequence in the vector genome is 0.4 kb.

[0196] In some embodiments, the viral genome is a self-complementary (sc) viral genome and comprises one or more filler sequences that, independently or together, have a length about

between 0.1 kb – 1.5 kb, such as, but not limited to, 0.1 kb, 0.2 kb, 0.3 kb, 0.4 kb, 0.5 kb, 0.6 kb, 0.7 kb, 0.8 kb, 0.9 kb, 1 kb, 1.1 kb, 1.2 kb, 1.3 kb, 1.4 kb, or 1.5 kb. In some embodiments, the total length filler sequence in the vector genome is 0.8 kb. In some embodiments, the total length filler sequence in the vector genome is 0.4 kb. In some embodiments, the length of each filler sequence in the vector genome is 0.8 kb. In some embodiments, the length of each filler sequence in the vector genome is 0.4 kb.

[0197] In some embodiments, the viral genome comprises any portion of a filler sequence. The viral genome may comprise 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of a filler sequence.

[0198] In some embodiments, the viral genome is a single stranded (ss) viral genome and comprises one or more filler sequences in order to have the length of the viral genome be about 4.6 kb. In some embodiments, the viral genome comprises at least one filler sequence and the filler sequence is located 3' to the 5' ITR sequence. In some embodiments, the viral genome comprises at least one filler sequence and the filler sequence is located 5' to a promoter sequence. In some embodiments, the viral genome comprises at least one filler sequence and the filler sequence is located 3' to the polyadenylation signal sequence. In some embodiments, the viral genome comprises at least one filler sequence and the filler sequence is located 5' to the 3' ITR sequence. In some embodiments, the viral genome comprises at least one filler sequence, and the filler sequence is located between two intron sequences. In some embodiments, the viral genome comprises at least one filler sequence, and the filler sequence is located within an intron sequence. In some embodiments, the viral genome comprises two filler sequences, and the first filler sequence is located 3' to the 5' ITR sequence and the second filler sequence is located 3' to the polyadenylation signal sequence. In some embodiments, the viral genome comprises two filler sequences, and the first filler sequence is located 5' to a promoter sequence and the second filler sequence is located 3' to the polyadenylation signal sequence. In some embodiments, the viral genome comprises two filler sequences, and the first filler sequence is located 3' to the 5' ITR sequence and the second filler sequence is located 5' to the 5' ITR sequence.

[0199] In some embodiments, the viral genome is a self-complementary (sc) viral genome and comprises one or more filler sequences in order to have the length of the viral genome be about 2.3 kb. In some embodiments, the viral genome comprises at least one filler sequence and the filler sequence is located 3' to the 5' ITR sequence. In some embodiments, the viral genome comprises at least one filler sequence and the filler sequence is located 5' to a promoter sequence. In some embodiments, the viral genome comprises at least one filler sequence and the

filler sequence is located 3' to the polyadenylation signal sequence. In some embodiments, the viral genome comprises at least one filler sequence and the filler sequence is located 5' to the 3' ITR sequence. In some embodiments, the viral genome comprises at least one filler sequence, and the filler sequence is located between two intron sequences. As a non-limiting example, the viral genome comprises at least one filler sequence, and the filler sequence is located within an intron sequence. In some embodiments, the viral genome comprises two filler sequences, and the first filler sequence is located 3' to the 5' ITR sequence and the second filler sequence is located 3' to the polyadenylation signal sequence. In some embodiments, the viral genome comprises two filler sequences, and the first filler sequence is located 5' to a promoter sequence and the second filler sequence is located 3' to the polyadenylation signal sequence. In some embodiments, the viral genome comprises two filler sequences, and the first filler sequence is located 3' to the 5' ITR sequence and the second filler sequence is located 5' to the 5' ITR sequence.

[0200] In some embodiments, the viral genome may comprise one or more filler sequences between one of more regions of the viral genome. In some embodiments, the filler region may be located before a region such as, but not limited to, a payload region, an inverted terminal repeat (ITR), a promoter region, an intron region, an enhancer region, a polyadenylation signal sequence region, and/or an exon region. In some embodiments, the filler region may be located after a region such as, but not limited to, a payload region, an inverted terminal repeat (ITR), a promoter region, an intron region, an enhancer region, a polyadenylation signal sequence region, and/or an exon region. In some embodiments, the filler region may be located before and after a region such as, but not limited to, a payload region, an inverted terminal repeat (ITR), a promoter region, an intron region, an enhancer region, a polyadenylation signal sequence region, and/or an exon region.

[0201] In some embodiments, the viral genome may comprise one or more filler sequences that bifurcate(s) at least one region of the viral genome. The bifurcated region of the viral genome may comprise 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the of the region to the 5' of the filler sequence region. In some embodiments, the filler sequence may bifurcate at least one region so that 10% of the region is located 5' to the filler sequence and 90% of the region is located 3' to the filler sequence. In some embodiments, the filler sequence may bifurcate at least one region so that 20% of the region is located 5' to the filler sequence and 80% of the region is located 3' to the filler sequence. In some embodiments, the filler sequence may bifurcate at least one region so that 30% of the region is located 5' to the filler

sequence and 70% of the region is located 3' to the filler sequence. In some embodiments, the filler sequence may bifurcate at least one region so that 40% of the region is located 5' to the filler sequence and 60% of the region is located 3' to the filler sequence. In some embodiments, the filler sequence may bifurcate at least one region so that 50% of the region is located 5' to the filler sequence and 50% of the region is located 3' to the filler sequence. In some embodiments, the filler sequence may bifurcate at least one region so that 60% of the region is located 5' to the filler sequence and 40% of the region is located 3' to the filler sequence. In some embodiments, the filler sequence may bifurcate at least one region so that 70% of the region is located 5' to the filler sequence and 30% of the region is located 3' to the filler sequence. In some embodiments, the filler sequence may bifurcate at least one region so that 80% of the region is located 5' to the filler sequence and 20% of the region is located 3' to the filler sequence. In some embodiments, the filler sequence may bifurcate at least one region so that 90% of the region is located 5' to the filler sequence and 10% of the region is located 3' to the filler sequence.

[0202] In some embodiments, the viral genome comprises a filler sequence after the 5' ITR.

[0203] In some embodiments, the viral genome comprises a filler sequence after the promoter region. In some embodiments, the viral genome comprises a filler sequence after the payload region. In some embodiments, the viral genome comprises a filler sequence after the intron region. In some embodiments, the viral genome comprises a filler sequence after the enhancer region. In some embodiments, the viral genome comprises a filler sequence after the polyadenylation signal sequence region. In some embodiments, the viral genome comprises a filler sequence after the exon region.

[0204] In some embodiments, the viral genome comprises a filler sequence before the promoter region. In some embodiments, the viral genome comprises a filler sequence before the payload region. In some embodiments, the viral genome comprises a filler sequence before the intron region. In some embodiments, the viral genome comprises a filler sequence before the enhancer region. In some embodiments, the viral genome comprises a filler sequence before the polyadenylation signal sequence region. In some embodiments, the viral genome comprises a filler sequence before the exon region.

[0205] In some embodiments, the viral genome comprises a filler sequence before the 3' ITR.

[0206] In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the 5' ITR and the promoter region. In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the 5' ITR and the payload region. In some embodiments, a filler sequence may be located between two regions, such as,

but not limited to, the 5' ITR and the intron region. In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the 5' ITR and the enhancer region. In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the 5' ITR and the polyadenylation signal sequence region.

[0207] In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the 5' ITR and the exon region.

[0208] In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the promoter region and the payload region. In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the promoter region and the intron region. In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the promoter region and the enhancer region. In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the promoter region and the polyadenylation signal sequence region. In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the promoter region and the exon region. In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the promoter region and the 3' ITR.

[0209] In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the payload region and the intron region. In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the payload region and the enhancer region. In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the payload region and the polyadenylation signal sequence region. In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the payload region and the exon region.

[0210] In some embodiments, a filler sequence may be located between two regions, such as, but not limited to, the payload region and the 3' ITR.

Viral Genome Component: Payloads

[0211] In some embodiments, the disclosure provides an AAV particle comprising a viral genome encoding a GBA1 protein, e.g., a GCase protein, encoded by the nucleotide sequences of SEQ ID NO: 2001 or SEQ ID NO: 2002. In some embodiments, the viral genome comprises a promoter operably linked to a nucleotide sequence encoding a GBA1 protein, e.g., SEQ ID NO: 2001 or SEQ ID NO: 2002. In some embodiments, the viral genome comprises the nucleotide sequence of SEQ ID NO: 2002.

[0212] In some embodiments, the disclosure herein provides constructs that allow for improved expression of GCase protein delivered by gene therapy vectors.

[0213] In some embodiments, the disclosure provides constructs that allow for improved biodistribution of GCase protein delivered by gene therapy vectors.

[0214] In some embodiments, the disclosure provides constructs that allow for improved sub-cellular distribution or trafficking of GCase protein delivered by gene therapy vectors.

[0215] In some embodiments, the disclosure provides constructs that allow for improved trafficking of GCase protein to lysosomal membranes delivered by gene therapy vectors.

[0216] In some embodiments, the present disclosure relates to a composition containing or comprising a nucleic acid sequence encoding a GBA1 protein or a functional fragment or variant thereof and methods of administering the composition *in vitro* or *in vivo* in a subject, e.g., a human subject and/or an animal model of disease, e.g., a disease related to expression of GBA.

[0217] AAV particles of the present disclosure may comprise a nucleic acid sequence encoding at least one “payload.” As used herein, “payload” or “payload region” refers to one or more polynucleotides or polynucleotide regions encoded by or within a viral genome or an expression product of such polynucleotide or polynucleotide region, e.g., a transgene, a polynucleotide encoding a polypeptide or multi-polypeptide, e.g., a GBA1 protein or a functional fragment or variant thereof. The payload may comprise any nucleic acid known in the art that is useful for the expression (by supplementation of the protein product or gene replacement using a modulatory nucleic acid) of a GBA1 protein in a target cell transduced or contacted with the AAV particle carrying the payload.

[0218] In some embodiments, the disclosure provides a nucleotide sequence encoding a GBA1 protein for use in an AAV genome, wherein the nucleotide sequence comprises a codon-optimized, CpG-reduced (e.g., CpG-depleted) GBA1-encoding sequence. In some embodiments, the CpG-reduced (e.g., CpG-depleted) GBA1-encoding sequence provides improved toxicity *in vivo*, e.g., reduced immunogenicity in a human or animal subject. In some embodiments, the nucleotide sequence further comprises one or more, e.g., all of, a 5' ITR sequence, a CMVie sequence, a CB promoter sequence, an intron sequence, a signal sequence, a polyA sequence, and a 3' ITR sequence. In some embodiments, the GBA1 protein encoded by the nucleotide sequence has an amino acid sequence that is 100% identical to a wildtype GBA1 protein. In some embodiments, the wildtype GBA1-encoding sequence is as provided by NCBI Reference Sequence NCBI Reference Sequence NP_000148.2 (SEQ ID NO: 14 of Int'l Pub. No. WO2019070893, incorporated by reference herein).

[0219] In some embodiments, the AAV genome encodes a payload construct that comprises a combination of coding and non-coding nucleic acid sequences.

[0220] In some embodiments, the viral genome encodes more than one payload. As a non-

limiting example, a viral genome encoding more than one payload may be replicated and packaged into a viral particle. A target cell transduced with a viral particle comprising more than one payload may express each of the payloads in a single cell.

[0221] In some embodiments, the viral genome may encode a coding or non-coding RNA. In certain embodiments, the adeno-associated viral vector particle further comprises at least one cis-element selected from the group consisting of a Kozak sequence, a backbone sequence, and an intron sequence.

[0222] In some embodiments, the payload is a polypeptide comprising a secreted protein, an intracellular protein, an extracellular protein, and/or a membrane protein. In some embodiments, the encoded proteins may be structural or functional. In some embodiments, the proteins encoded by the viral genome include, but are not limited to, mammalian proteins. In certain embodiments, the AAV particle comprises a viral genome that encodes GBA1 protein or a functional fragment or variant thereof. The AAV particles described herein may be useful in the fields of human disease, veterinary applications, and a variety of *in vivo* and *in vitro* settings.

[0223] In some embodiments, a payload comprises a polypeptide that serve as a marker protein to assess cell transformation and expression, a fusion having a desired biological activity, a gene product that can complement a genetic defect, an RNA molecule, a transcription factor, and/or another gene products related to gene regulation and/or expression.

[0224] In some embodiments, the payload comprises a gene therapy product including, but not limited to, a polypeptide, RNA molecule, or other gene product that, when expressed in a target cell, provides a desired therapeutic effect. In some embodiments, a gene therapy product may comprise a substitute for a non-functional gene or a gene that is absent, expressed in insufficient amounts, or mutated. In some embodiments, a gene therapy product may comprise a substitute for a non-functional protein or polypeptide or a protein or polypeptide that is absent, expressed in insufficient amounts, misfolded, degraded too rapidly, or mutated. For example, a gene therapy product may comprise a polynucleotide encoding a GBA1 protein to treat GCase deficiency or GBA1-related disorders. In some embodiments, the gene therapy product comprises a polynucleotide sequence encoding a GBA1 protein.

[0225] In some embodiments, the payload encodes a messenger RNA (mRNA). As used herein, the term “messenger RNA” (mRNA) refers to any polynucleotide that encodes a polypeptide of interest and that is capable of being translated to produce the encoded polypeptide of interest *in vitro*, *in vivo*, *in situ*, or *ex vivo*. Certain embodiments provide the mRNA as encoding GCase or a variant thereof.

[0226] In some embodiments, the protein or polypeptide encoded by the payload construct

encoding GCase or a functional variant thereof is between about 50 and about 4500 amino acid residues in length (hereinafter in this context, “X amino acids in length” refers to X amino acid residues). In some embodiments, the protein or polypeptide encoded is between 50-2000 amino acids in length. In some embodiments, the protein or polypeptide encoded is 50-1000 amino acids in length. In some embodiments, the protein or polypeptide encoded is 50-1500 amino acids in length. In some embodiments, the protein or polypeptide encoded is 50-1000 amino acids in length. In some embodiments, the protein or polypeptide encoded is 50-800 amino acids in length. In some embodiments, the protein or polypeptide encoded is 50-600 amino acids in length. In some embodiments, the protein or polypeptide encoded is 50-400 amino acids in length. In some embodiments, the protein or polypeptide encoded is 50-200 amino acids in length. In some embodiments, the protein or polypeptide encoded is 50-100 amino acids in length. In some embodiments, the protein or polypeptide encoded is 497 amino acids in length.

[0227] A payload construct encoding a payload may comprise or encode a selectable marker. A selectable marker may comprise a gene sequence or a protein or polypeptide encoded by a gene sequence expressed in a host cell that allows for the identification, selection, and/or purification of the host cell from a population of cells that may or may not express the selectable marker. In some embodiments, the selectable marker provides resistance to survive a selection process that would otherwise kill the host cell, such as treatment with an antibiotic. In some embodiments, an antibiotic selectable marker may comprise one or more antibiotic resistance factors, including but not limited to neomycin resistance (*e.g.*, neo), hygromycin resistance, kanamycin resistance, and/or puromycin resistance.

[0228] In some embodiments, a payload construct encoding a payload may comprise a selectable marker including, but not limited to, β -lactamase, luciferase, β -galactosidase, or any other reporter gene as that term is understood in the art, including cell-surface markers, such as CD4 or the truncated nerve growth factor (NGFR) (for GFP, *see* WO 96/23810; Heim *et al.*, *Current Biology* 2:178-182 (1996); Heim *et al.*, *Proc. Natl. Acad. Sci. USA* (1995); or Heim *et al.*, *Science* 373:663-664 (1995); for β -lactamase, *see* WO 96/30540); the contents of each of which are herein incorporated by reference in their entirety.

[0229] In some embodiments, a payload construct encoding a selectable marker may comprise a fluorescent protein. A fluorescent protein as herein described may comprise any fluorescent marker including but not limited to green, yellow, and/or red fluorescent protein (GFP, YFP, and/or RFP). In some embodiments, a payload construct encoding a selectable marker may comprise a human influenza hemagglutinin (HA) tag.

[0230] In certain embodiments, a nucleic acid for expression of a payload in a target cell will

be incorporated into the viral genome and located between two ITR sequences.

[0231] In some embodiments, a payload construct further comprises a nucleic acid sequence encoding a peptide that binds to the cation-independent mannose 6- phosphate (M6P) receptor (CI-MPR) with high affinity, as described in Int’l Pat. App. Pub. No. WO2019213180A1, the disclosure of which is incorporated herein by reference in its entirety. The peptide that binds CI-MPR can be, *e.g.*, an IGF2 peptide or variant thereof. Binding of CI-MPR can facilitate cellular uptake or delivery and intracellular or sub-cellular targeting of therapeutic proteins provided by gene therapy vectors.

Payload Component: Signal Sequence

[0232] In some embodiments, the nucleic acid sequence comprising the transgene encoding the payload, *e.g.*, a GBA1 protein, comprises a nucleic acid sequence encoding a signal sequence (*e.g.*, a signal sequence region herein).

[0233] In some embodiments, the nucleotide sequence encoding the signal sequence is located 5’ relative to the nucleotide sequence encoding the GBA1 protein. In some embodiments, the encoded GBA1 protein comprises a signal sequence at the N-terminus, wherein the signal sequence is optionally cleaved during cellular processing and/or localization of the GBA1 protein and/or the enhancement element.

[0234] In some embodiments, the signal sequence comprises SEQ ID NO: 2005 or a sequence that is at least 85% identical (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto. In some embodiments, the signal sequence comprises the amino acid sequence of SEQ ID NO: 1853 or an amino acid sequence at least at least 90% identical (*e.g.*, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

Exemplary GCase (GBA1) Protein Payload

[0235] In some embodiments, the payload, *e.g.*, of a viral genome described herein, is a wildtype GBA1 protein, *e.g.*, a wild-type GBA1 protein.

[0236] Tables 2A and 2B provide exemplary polynucleotide sequences encoding a GBA1 protein and polypeptide sequences of exemplary GBA1 proteins that may be used in the viral genomes disclosed herein and which may constitute a GBA1 protein payload. In some embodiments, the GBA1 protein suitable for delivery in an AAV disclosed herein is encoded by the nucleotide sequence of SEQ ID NO: 2001 or SEQ ID NO: 2002.

Table 2A. Exemplary GCase Sequences

SEQ ID NO:	Type	Species	Description
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1740	Protein	<i>Homo sapiens</i>	GBA1 protein NP_000148.2
1741	DNA	<i>Homo sapiens</i>	GBA1 mRNA transcript variant 1 NM_000157.4
1742	Protein	<i>Homo sapiens</i>	GBA1 protein NP_001005741.1
1743	DNA	<i>Homo sapiens</i>	GBA1 mRNA transcript variant 2 NM_01005741.3
1744	Protein	<i>Homo sapiens</i>	GBA1 protein NP_001005742.1
1745	DNA	<i>Homo sapiens</i>	GBA1 mRNA transcript variant 3 NM_001005742.3
1746	Protein	<i>Homo sapiens</i>	GBA1 protein NP_001165282.1
1747	DNA	<i>Homo sapiens</i>	GBA1 mRNA transcript variant 4 NM_001171811.2
1748	Protein	<i>Homo sapiens</i>	GBA1 protein NP_001165283.1
1749	DNA	<i>Homo sapiens</i>	GBA1 mRNA transcript variant 5 NM_001171812.2

Table 2B. Exemplary GCase Sequences

Description	Sequence	SEQ ID NO:
GBA1 Variant 1 (signal sequence underlined)-nt	<u>ATGGAATTCTCTAGCCCATCTAGAGAGGAATGTCCTAAGCCTCTGTCAA</u> <u>GAGTGTCCATCATGGCCGGCAGCCTGACAGGCCTGCTGCTGCTGCAGGC</u> <u>CGTGTCTCTGGGCCAGTGGAGCCCGGCCCTGCATCCCTAAGTCCTTCGGC</u> <u>TATTCTAGCGTGGTCTGCGTGTGTAATGCCACTTACTGCGACAGCTTCG</u> <u>ACCCTCCTACCTTCCCCGCCCTTGGAACTGAGCATGGGCCCAATCCAGGCCAAC</u> <u>CAGAAGCGGCAGAAGAATGGAAGTGAACATTCAGCAGATACGAGAGCAC</u> <u>CACACCGGCACCGGCCCTGCTGCTGACACTGCAACCTGAGCAGAAGTTC</u> <u>AGAAGGTGAAGGGATTTGGAGGCGCCATGACCGACGCTGCTGCTCTGAA</u> <u>CATCCTGGCCCTCTCCCCACCTGCTCAGAACCTGCTGCTTAAAAGCTAC</u> <u>TTCAGCGAGGAAGGCATCGGCTATAACATCATCAGAGTGCCCATGGCCA</u> <u>GCTGCGACTTCAGCATCAGAACATACACCTACGCCGATACACCTGATGA</u> <u>CTTCCAACCTGCACAACCTTCAGCCTGCCTGAAGAGGACACAAAGCTGAAA</u> <u>ATCCCCCTGATCCACCGGGCCCTGCAGCTGGCCCGAGAGACCTGTGAGCC</u> <u>TGCTGGCCTCTCCTTGGACAAGCCCCACCTGGCTGAAGACCAATGGAGC</u> <u>TGTGAACGGCAAGGGCAGCCTGAAGGGCCAGCCCGGCAGCATCTACCAC</u> <u>CAAACCTGGGCTCGCTACTTCGTGAAATTCCTGGACGCCTACGCTGAGC</u> <u>ATAAGCTGCAATTTTGGGCCGTTACAGCCGAGAACGAGCCTTCTGCCGG</u> <u>CCTGCTGTCTGGATATCCTTTCCAGTGCCTGGGCTTCACCCCTGAGCAC</u> <u>CAGAGAGACTTTATCGCCAGAGATCTGGGGCCTACCCTGGCTAACAGCA</u> <u>CACACCACAACGTGCGGCTGCTGATGCTGGACGATCAGAGGCTGCTGCT</u> <u>CCCCCACTGGGCCAAGGTGGTGTGACAGATCCGGAGGCCGCCAAATAC</u> <u>GTGCACGGCATCGCCGTCCACTGGTACCTGGATTTCTGGCCCCCTGCCA</u> <u>AGGCCACCCTGGGCGAGACACATAGACTGTTTCTTAATACCATGCTGTT</u> <u>CGCCAGCGAGGCCTGCGTGGGCAGCAAGTTCTGGGAACAGAGCGTGCGG</u> <u>CTGGGCAGCTGGGACAGAGGAATGCAGTACAGCCACAGCATCATTACCA</u> <u>ACCTGCTGTACCAGTGGTGGGCTGGACCGACTGGAACCTGGCCCTGAA</u> <u>CCCCGAAGGCGGCCCAACTGGGTGCGGAACTTCGTGGACTCTCTCATC</u> <u>ATCGTGGATATTACCAAGGATACCTTTTACAAGCAGCCTATGTTCTACC</u> <u>ACCTGGGCCACTTCAGCAAGTTCATCCCTGAGGGCTCTCAGCGGGTGGG</u> <u>CCTGGTGGCCTCTCAGAAAAACGACCTGGATGCCGTTGCCCTGATGCAC</u> <u>CCCGACGGCAGCGCCGTGGTGGTCTGCTGAATAGAAGCTCCAAGGACG</u> <u>TGCCTCTGACCATCAAGGACCCCGCTGTGGGATTTCTGGAAACCATCAG</u> <u>CCCTGGCTACAGCATCCACACCTACCTGTGGCGCGGCAG</u>	1772

GBA1 Variant 1 (no signal sequence)-nt	GCCCGGCCCTGCATCCCTAAGTCCTTCGGCTATTCTAGCGTGGTCTGCG TGTGTAATGCCACTTACTGCGACAGCTTCGACCCTCCTACCTTCCCCGC CCTTGGAACATTTCAGCAGATACGAGAGCACCAGAAGCGGCAGAAGAATG GAACTGAGCATGGGCCCCAATCCAGGCCAACCACACCGGCACCGGCCTGC TGCTGACACTGCAACCTGAGCAGAAGTTCCAGAAGGTGAAGGGATTTGG AGGCGCCATGACCGACGCTGCTGCTCTGAACATCCTGGCCCTCTCCCCA CCTGCTCAGAACCTGCTGCTTAAAAGCTACTTCAGCGAGGAAGGCATCG GCTATAACATCATCAGAGTGCCCATGGCCAGCTGCGACTTCAGCATCAG AACATAACACCTACGCCGATACACCTGATGACTTCCAATGCACAACCTTC AGCCTGCCTGAAGAGGACACAAAGCTGAAAATCCCCCTGATCCACCGGG CCCTGCAGCTGGCCAGAGACCTGTGAGCCTGCTGGCCTCTCCTTGGAC AAGCCCCACCTGGCTGAAGACCAATGGAGCTGTGAACGGCAAGGGCAGC CTGAAGGGCCAGCCCGGCACATCTACCACCAAACCTGGGCTCGCTACT TCGTGAAATTCTGGACGCCCTACGCTGAGCATAAGCTGCAATTTTGGGC CGTTACAGCCGAGAACGAGCCTTCTGCCGGCCTGCTGTCTGGATATCCT TTCCAGTGCTGGGCTTCACCCCTGAGCACCAGAGAGACTTTATCGCCA GAGATCTGGGGCCTACCCTGGCTAACAGCACACACCACAACGTGCGGCT GCTGATGCTGGACGATCAGAGGCTGCTGCTCCCCCACTGGGCCAAGGTG GTGCTGACAGATCCGGAGGCCGCCAAATACGTGCACGGCATCGCCGTCC ACTGGTACCTGGATTTCCTGGCCCCCTGCCAAGGCCACCCTGGGCGAGAC ACATAGACTGTTTCCTAATACCATGCTGTTGCCAGCGAGGCCTGCGTG GGCAGCAAGTTCTGGGAACAGAGCGTGCGGCTGGGCAGCTGGGACAGAG GAATGCAGTACAGCCACAGCATCATTAACCAACCTGCTGTACCACGTGGT GGGCTGGACCGACTGGAACCTGGCCCTGAACCCCGAAGGCGGCCCAAC TGGGTGCGGAACCTTCGTGGACTCTCCTATCATCGTGGATATTACCAAGG ATACCTTTTACAAGCAGCCTATGTTCTACCACCTGGGCCACTTCAGCAA GTTTCATCCCTGAGGGCTCTCAGCGGCTGGGCCTGGTGGCCTCTCAGAAA AACGACCTGGATGCCGTTGCCCTGATGCACCCCGACGGCAGCGCCGTGG TGGTCGTCCTGAATAGAAGCTCCAAGGACGTGCCTCTGACCATCAAGGA CCCCGCTGTGGGATTTCTGGAACCATCAGCCCTGGCTACAGCATCCAC ACCTACCTGTGGCGGCGGCAG	1773
GBA1 Variant 1 (signal sequence underlined)-aa	<u>MEFSSPSREEC</u>PKPLSRV<u>SIMAGSLTGLLLLQAVSWASGARPC</u>IPKSF GYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELSMGPIQAN HTGTGLLLTLQPEQKFQVKGFGGAMTDAAALNILALSPPAQNLLLSY FSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLK IPLIHRALQLAQRPVSLASPWTSPWLKTNGAVNGKSLKGQPGDIYH QWTWARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEH QRDFIARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDPEAAKY VHGIAVHWYLDFLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVR LGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPI IVDITKDTFYKQPMFYHLGHFSKFIPEGSQVRGLVASQKNDLDAVALMH PDGSAVVVVLNRSSKDVPLTIKDPAVGFLETISPGYSIHTYLWRRQ	1774
GBA1 Variant 1 (no signal sequence)-aa	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRM ELSMGPIQANHTGTGLLLTLQPEQKFQVKGFGGAMTDAAALNILALSP PAQNLLLSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNF SLPEEDTKLKIPLIHRALQLAQRPVSLASPWTSPWLKTNGAVNGKGS LKGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYP FQCLGFTPEHQRDFIARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKV VLTDPEAAKYVHGIAVHWYLDFLAPAKATLGETHRLFPNTMLFASEACV GSKFWEQSVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEGGPW WVRNFVDSPIIVDITKDTFYKQPMFYHLGHFSKFIPEGSQVRGLVASQK NDLDAVALMHPDGSAVVVVLNRSSKDVPLTIKDPAVGFLETISPGYSIHTYLWRRQ	1775

GBA1 Variant 2 (signal sequence underlined)-nt	ATGGAGTTTTCAAGTCCTTCCAGAGAGGAATGTCCCAAGCCTTTGAGTA GGGTAAGCATCATGGCTGGCAGCCTCACAGGATTGCTTCTACTTCAGGC <u>AGTGTCTGTGGGCATCAGGTGCCCCGCCCTGCATCCCTAAAAGCTTCGGC</u> TACAGCTCGGTGGTGTGTGTCTGCAATGCCACATACTGTGACTCCTTTG ACCCCCGACCTTTCTGCCCCTTGGTACCTTCAGCCGCTATGAGAGTAC ACGCAGTGGGCGACGGATGGAGCTGAGTATGGGGCCCATCCAGGCTAAT CACACGGGCACAGGCCTGCTACTGACCCTGCAGCCAGAACAGAAGTTCC AGAAAGTGAAGGGATTTGGAGGGGCCATGACAGATGCTGCTGCTCTCAA CATCCTTGCCCTGTCACCCCTGCCAAAATTTGCTACTTAAATCGTAC TTCTCTGAAGAAGGAATCGGATATAACATCATCCGGGTACCCATGGCCA GCTGTGACTTCTCCATCCGCACCTACACCTATGCAGACACCCCTGATGA TTTCCAGTTGCACAACCTTCAGCCTCCCAGAGGAAGATACCAAGCTCAAG ATACCCCTGATTACCGAGCCCTGCAGTTGGCCCAGCGTCCCGTTTCAC TCCTTGCCAGCCCTGGACATCACCCACTTGGCTCAAGACCAATGGAGC GGTGAATGGGAAGGGGTCACTCAAGGGACAGCCCGGAGACATCTACCAC CAGACCTGGGCCAGATACTTTGTGAAGTTCCTGGATGCCTATGCTGAGC ACAAGTTACAGTTCTGGGCAGTGACAGCTGAAAATGAGCCTTCTGCTGG GCTGTTGAGTGGATAACCCCTTCCAGTGCCTGGGCTTCACCCCTGAACAT CAGCGAGACTTCATTGCCCGTGACCTAGGTCTACCCCTCGCCAACAGTA CTCACCACAATGTCCGCCCTACTCATGCTGGATGACCAACGCTTGCTGCT GCCCCACTGGGCAAAGGTGGTACTGACAGACCCAGAAGCAGCTAAATAT GTTTCATGGCATTGCTGTACATTGGTACCTGGACTTCTGGCTCCAGCCA AAGCCACCCTAGGGGAGACACACCGCCTGTTCCCCAACACCATGCTCTT TGCCTCAGAGGCCTGTGTGGGCTCCAAGTTCTGGGAGCAGAGTGTGCGG CTAGGCTCCTGGGATCGAGGGATGCAGTACAGCCACAGCATCATCACGA ACCTCCTGTACCATGTGGTTCGGCTGGACCGACTGGAACCTTGCCCTGAA CCCCGAAGGAGGACCAATTGGGTGCGTAACTTTGTGACAGTCCCATC ATTGTAGACATCACCAAGGACACGTTTACAAACAGCCCATGTTCTACC ACCTTGCCCACTTCAGCAAGTTTCATTCCTGAGGGCTCCCAGAGAGTGGG GCTGGTTGCCAGTCAGAAGAAGACCTGGACGCAGTGGCACTGATGCAT CCCGATGGCTCTGCTGTTGTGGTCGTGCTAAACCGCTCCTCTAAGGATG TGCCTCTTACCATCAAGGATCCTGCTGTGGGCTTCCTGGAGACAATCTC ACCTGGCTACTCCATTACACCTACCTGTTGGCGTCGCCAG	1776
GBA1 Variant 2 (no signal sequence)-nt	GCCCCCCCCTGCATCCCTAAAAGCTTCGGCTACAGCTCGGTGGTGTGTG TCTGCAATGCCACATACTGTGACTCCTTTGACCCCCGACCTTTCTGCTG CCTTGGTACCTTCAGCCGCTATGAGAGTACACGCAGTGGGCGACGGATG GAGCTGAGTATGGGGCCCATCCAGGCTAATCACACGGGCACAGGCCTGC TACTGACCCTGCAGCCAGAACAGAAGTTCAGAAAGTGAAGGGATTTGG AGGGGCCATGACAGATGCTGCTGCTCTCAACATCCTTGCCCTGTCACCC CCTGCCCCAAAATTTGCTACTTAAATCGTACTTCTCTGAAGAAGGAATCG GATATAACATCATCCGGGTACCCATGGCCAGCTGTGACTTCTCCATCCG CACCTACACCTATGCAGACACCCCTGATGATTTCCAGTTGCACAACCTTC AGCCTCCCAGAGGAAGATACCAAGCTCAAGATACCCCTGATTACCCGAG CCCTGCAGTTGGCCAGCGTCCCGTTTCACTCCTTGCCAGCCCTGGAC ATCACCCACTTGGCTCAAGACCAATGGAGCGGTGAATGGGAAGGGGTCA CTCAAGGGACAGCCCGGAGACATCTACCACCAGACCTGGGCCAGATACT TTGTGAAGTTCTGGATGCCTATGCTGAGCACAAGTTACAGTTCTGGGC AGTGACAGCTGAAAATGAGCCTTCTGCTGGGCTGTTGAGTGGATACCCC TTCCAGTGCCTGGGCTTCACCCCTGAACATCAGCGAGACTTCATTGCCC GTGACCTAGGTCTACCCCTCGCCAACAGTACTCACCACAATGTCCGCCT ACTCATGCTGGATGACCAACGCTTGCTGCTGCCCCACTGGGCAAAGGTG GTACTGACAGACCCAGAAGCAGCTAAATATGTTTCATGGCATTGCTGTAC ATTGGTACCTGGACTTTCTGGCTCCAGCCAAAGCCACCCTAGGGGAGAC ACACCGCCTGTTCCCCAACACCATGCTCTTTGCCTCAGAGGCCTGTGTG GGCTCCAAGTTCTGGGAGCAGAGTGTGCGGCTAGGCTCCTGGGATCGAG	1777

	GGATGCAGTACAGCCACAGCATCATCACGAACCTCCTGTACCATGTGGT CGGCTGGACCGACTGGAACCTTGCCCTGAACCCGAAGGAGGACCCAAT TGGGTGCGTAACTTTGTTCGACAGTCCCATCATTGTAGACATCACCAAGG ACACGTTTTTACAAACAGCCCATGTTCTACCACCTTGGCCACTTCAGCAA GTTTCATTCCCTGAGGGCTCCCAGAGAGTGGGGCTGGTTGCCAGTCAGAAG AACGACCTGGACGCAGTGGCACTGATGCATCCCGATGGCTCTGCTGTTG TGGTCGTGCTAAACCGCTCCTCTAAGGATGTGCCTCTTACCATCAAGGA TCCTGCTGTGGGCTTCCTGGAGACAATCTCACCTGGCTACTCCATTAC ACCTACCTGTGGCGTCGCCAG	
GBA1 Variant 2 (signal sequence underlined)-aa	<u>MEFSSPSREEC</u> PKPLSRVSI <u>MAGSLTGLLLLQAVSWASGAR</u> PCIPKSF YSSVVCVCNATYCDSFDPTFPALGTFSTRSGRRMELSMGPIQAN HTGTGLLLTLQPEQKFQVKGFGGAMTDAAALNILALSPPAQNLLLSY FSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLK IPLIHRALQLAQRVSLASPWTSPWLKTNGAVNGKGS LKGQPGDIYH QTWARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEH QRDFIARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDPEAAKY VHGI AVHWYLD FLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVR LGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEGGPNWVRNFVDSPI IVDITKDTFYKQPMFYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMH PDGSAVVVVLNRSSKDVPLTIKDPAVGFLETISPGYSIHTYLWRRQ	1778
GBA1 Variant 2 (no signal sequence)-aa	ARPCIPKSFYSSVVCVCNATYCDSFDPTFPALGTFSTRSGRRM ELSMGPIQANHTGTGLLLTLQPEQKFQVKGFGGAMTDAAALNILALSP PAQNLLLSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNF SLPEEDTKLKIPLIHRALQLAQRVSLASPWTSPWLKTNGAVNGKGS LKGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYP FQCLGFTPEHQRDFIARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKV VLTDPEAAKYVHGI AVHWYLD FLAPAKATLGETHRLFPNTMLFASEACV GSKFWEQSVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEGGPN WVRNFVDSPIIVDITKDTFYKQPMFYHLGHFSKFIPEGSQRVGLVASQK NDLDAVALMHPDGSAVVVVLNRSSKDVPLTIKDPAVGFLETISPGYSIH TYLWRRQ	1779
GBA1 Variant 3 (signal sequence underlined)-nt	<u>atggaattcagcagccccagcagagaggaatgccccaa</u> gcctctgagcc <u>gggtgtcaatcatggcggatctctgacaggactgctgcttcaggc</u> <u>cgtgtcttgggcttctggcgctagaccttgcatccccaa</u> gagcttcggc tacagcagcgtcgtgtgctgtgcaatgccacctactgcgacagcttcg acctcctacctttctgctctgggcaccttcagcagatacagagcac cagatccggcagacggatggaactgagcatgggacctatccaggccaat cacacaggcactggcctgctgctgacactgcagcctgagcagaaattcc agaaagtgaaggttcggcggagccatgacagatgccgcccgtctgaa tattcctggctctgtctccaccagctcagaacctgctgctcaagagctac ttcagcaggaaggcatcggctacaacatcatcagagtgcccatggcca gctgcgacttcagcatcaggacctacacctacgccgacacacccgacga tttccagctgcacaacttcagcctgcctgaagaggacaccaagctgaag atccctctgatccacagagccctgcagctggcacaagacccgtgtcac tgctggcctctccatggacatctcccacctggctgaaaacaaatggcgc cgtgaatggcaaggcagcctgaaaggccaacctggcgacatctaccac cagacctgggcccagatacttcgtgaagttcctggacgcctatgccgagc acaagctgcagttttgggcccgtgacagccgagaacgaaccttctgctgg actgctgagcggctaccttctcagtgctgggctttacacccgagcac cagcgggactttatcgcccgatctgggacctacactggccaatagca cccaccataatgtgcggctgctgatgctggacgaccagagactgcttct gccccactgggctaagtgtgctgacagatcctgaggccgccaatac gtgcacggaatcgccgtgactggtatctggactttctggccccctgcca aggccacactgggagagacacacagactgttccccaacacctgctgtt	1780

	cgccagcgaagcctgtgtgggcagcaagttttgggaacagagcgtgcg ctcggcagctgggatagaggcatgcagtacagccacagcatcatcacca acctgctgtaccacgtcgtcggctggaccgactggaatctggccctgaa tcctgaaggcggccctaactgggtccgaaacttcgtggacagccccatc atcgtggacatcaccaaggacaccttctacaagcagcccatgttctacc acctgggacacttcagcaagttcatccccgagggctctcagcggttg actgggtggcttcccagaagaacgatctggacgcccgtggctctgatgcac cctgatggatctgctgtgggtgggtcctgaaccgcagcagcaaagatg tgccccctgaccatcaaggatcccgccgtgggattcctggaacaatcag ccctggctactccatccacacctacctgtggcgtagacag	
GBA1 Variant 3 (no signal sequence)-nt	gctagaccttgcatccccaaagagcttcggctacagcagcgtcgtgtgcg tgtgcaatgccacctactgcgacagcttcgaccctcctacctttcctgc tctgggcaccttcagcagatacagagcaccagatccggcagacggatg gaactgagcatgggacctccaggccaatcacacaggcactggcctgc tgctgacactgcagcctgagcagaaattccagaaagtgaagggttcgg cggagccatgacagatgccgcccgtctgaatatcctggctctgtctcca ccagctcagaacctgctgctcaagagctacttcagcgaggaaggcatcg gctacaacatcatcagagtgcccatggccagctgcgacttcagcatcag gacctacacctacgccgacacacccgacgatttccagctgcacaacttc agcctgcctgaagaggacaccaagctgaagatccctctgatccacagag ccctgcagctggcacaagacccgtgtcactgctggcctctccatggac atctcccacctggctgaaaacaaatggcgccgtgaatggcaagggcagc ctgaaaggccaacctggcgacatctaccaccagacctgggcccagatact tcgtgaagttcctggacgcctatgccgagcacaagctgcagttttgggc cgtgacagccgagaacgaaccttctgctggactgctgagcggctacccc tttcagtgcttgggctttacacccgagcaccagcgggactttatcgccc gtgatctgggacctgacactggccaatagcaccaccataatgtgcccgt gctgatgctggacgaccagagactgcttctgccccactgggctaaagtg gtgctgacagatcctgaggccgcaaatacgtgcacggaatcgccgtgc actggtatctggactttctggccccctgccaaaggccacactgggagagac acacagactgttccccaacacctatgctgttcgccagcgaagcctgtgtg ggcagcaagttttgggaacagagcgtgcggctcggcagctgggatagag gcatgcagtacagccacagcatcatcaccaacctgctgtaccacgtcgt cggctggaccgactggaatctggccctgaatcctgaaggcggccctaac tgggtccgaaacttcgtggacagccccatcatcgtggacatcaccaagg acaccttctacaagcagccccatgttctaccacctgggacacttcagcaa gttcatccccgagggctctcagcgcttggactggtggcttcccagaag aacgatctggacgcccgtggctctgatgcacctgatggatctgctgtgg tggtggctcctgaaccgcagcagcaaagatgtgccccctgaccatcaagga tccccgctgggattcctggaacaatcagccctggctactccatccac acctacctgtggcgtagacag	1781
GBA1 Variant 3 (signal sequence underlined)-aa	<u>MEFSSPSREEC</u> PKPLSRVSIMAGSLTGLLLLQAVSWASGARPCIPKSF YSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRMELSMGPIQAN HTGTGLLLTLQPEQKFQVKGFGGAMTDAAALNILALSPPAQNLLLSY FSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLK IPLIHRALQLAQRPVSLASPWTSPTWLKTNGAVNGKSLKGQPGDIYH QTWARYFVKFLDAYAEHLQFWAVTAENEPSAGLLSGYPFQCLGFTPEH QRDFIARDLGPTLANSTHHNVRLLMLDDQRLLLPHWAKVVLTDPEAAKY VHGIAVHWYLDLFLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVR LGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEGGPNNVRNFVDSPI IVDITKDTFYKQPMFYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMH PDGSAVVVVLNRSSKDVP LTIKDPVGFLETISP GYSIHTYLWRRQ	1782

GBA1 Variant 3 (no signal sequence)-aa	ARPCIPKSFYSSVVCVCNATYCDSEDPPTFPALGTFSTRYESTRSGRRM ELSMGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSP PAQNLLLSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNF SLPEEDTKLKIPLIHRALQLAQRPVSLASPWTSPWLKTNGAVNGKGS LKGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYP FQCLGFTPEHQRDFIARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKV VLTDPEAAKYVHGIAVHWYLDLFLAPAKATLGETHRLFPNTMLFASEACV GSKFWEQSVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEGPN WVRNFVDSPIIVDITKDTFYKQPMFYHLGHFSKFIPEGSQVRVLVASQK NDLDAVALMHPDGSASVVVVLNRSSKDVPLTIKDPVAVGFLETISPYSIH TYLWRRQ	1783
GBA1 Variant 4 (signal sequence underlined)-nt	ATGGAATTCTCTAGCCCATCTAGAGAGGAATGTCCTAAGCCTCTGTCAA GAGTGTCCATCATGGCTGGCAGCCTGACAGGCCTGCTGCTGCTGCAGGC TGTGTCTCTGGCCAGTGGAGCCAGGCCCTGCATCCCTAAGTCCTTTGGC TATTCTTCTGTGGTCTGTGTGTGTAATGCCACTTACTGTGACAGCTTTG ACCCCTCCTACCTTCCCTGCCCTTGGACATTCAGCAGATATGAGAGCAC CAGATCTGGCAGAAGAATGGAACCTGAGCATGGGCCCAATCCAGGCCAAC CACACAGGCACAGGCCTCCTGCTGACACTGCAACCTGAGCAGAAGTTCC AGAAGGTGAAGGGATT'TGGAGGTGCCATGACAGATGCTGCTGCTCTGAA CATCCTGGCCCTCTCCCCACCTGCTCAGAACCTGCTGCTTAAAAGCTAC TTCTCTGAGGAAGGCATTGGCTATAACATCATCAGAGTGCCCATGGCCA GCTGTGACTTCAGCATCAGAACATACACTTATGCTGATACACCTGATGA CTTCCAACCTGCACAACCTTCAGTCTGCCTGAAGAGGACACAAAGCTGAAA ATCCCCCTGATCCACAGGGCCCTCCAGCTGGCCCAAGAGACCTGTGAGCC TGCTGGCCTCTCCTTGGACAAGCCCCACCTGGCTGAAGACTAATGGAGC TGTGAATGGCAAGGGCAGCCTGAAGGGCCAGCCTGGGGACATCTACCAC CAAACCTGGGCTAGGTACTTTGTCAAATTCCTGGATGCCTATGCTGAGC ATAAGCTGCAATTTTGGGCTGTTACAGCTGAGAATGAGCCTTCTGCAGG CCTGCTGTCTGGATATCCTTTCCAGTGCCTGGGCTTCACCCCTGAGCAC CAGAGAGACTTTAT'TGCCAGAGATCTGGGGCCAACCCTGGCTAACAGCA CACACCACAATGTGAGGCTGCTGATGCTGGATGACCAGAGGCTGCTGCT CCCCCACTGGGCCAAGGTGGTGTGCTGACAGATCCAGAGGCTGCCAAATAT GTGCATGGGATTGCTGTCCACTGGTACCTGGATTTCCTGGCCCCCTGCCA AGGCCACCCTGGGAGAAACACATAGACTGTTTCTTAATACCATGCTGTT TGCCTCTGAGGCCTGTGTGGGCAGCAAGTTCTGGGAACAATCTGTGAGA CTGGGCAGCTGGGACAGAGGAATGCAGTACAGCCACAGCATCATTACCA ACCTGCTGTATCATGTGGTGGGCTGGACAGACTGGAACCTGGCCCTCAA CCCTGAAGGGGGCCCCAACTGGGTGAGGAACCTTTGTGGACTCTCCTATC ATTGTGGATATTACCAAGGATACCTTCTACAAGCAGCCTATGTTCTACC ACCTGGGCCACTTCAGCAAGTTCATCCAGAGGGATCTCAGAGGGTGGG CCTTGTGGCATCTCAGAAAAATGACCTAGATGCTGTTGCCCTGATGCAC CCTGATGGGTGAGCAGTGGTAGTGGTCCTGAATAGAAGCTCCAAGGATG TGCCACTGACTATCAAGGACCCAGCTGTAGGATTTCTGGAAACCATCAG TCCTGGGTACAGCATCCACACTTACCTCTGGAGGAGGCAG	2001
GBA1 Variant 4 (no signal sequence)-nt	GCCAGGCCCTGCATCCCTAAGTCCTTTGGCTATTCTTCTGTGGTCTGTG TGTGTAATGCCACTTACTGTGACAGCTTTGACCCTCCTACCTTCCCTGC CCTTGGAACATTTCAGCAGATATGAGAGCACCAGATCTGGCAGAAGAATG GAACTGAGCATGGGCCCAATCCAGGCCAACACACAGGCACAGGCCTCC TGCTGACACTGCAACCTGAGCAGAAGTTCCAGAAGGTGAAGGGATTGG AGGTGCCATGACAGATGCTGCTGCTCTGAACATCCTGGCCCTCTCCCCA CCTGCTCAGAACCTGCTGCTTAAAAGCTACTTCTCTGAGGAAGGCATTG GCTATAACATCATCAGAGTGCCCATGGCCAGCTGTGACTTCAGCATCAG AACATACACTTATGCTGATACACCTGATGACTTCCAACCTGCACAACCTTC AGTCTGCCTGAAGAGGACACAAAGCTGAAAAATCCCCCTGATCCACAGGG CCCTCCAGCTGGCCCAGAGACCTGTGAGCCTGCTGGCCTCTCCTTGGAC	2002

	AAGCCCCACCTGGCTGAAGACTAATGGAGCTGTGAATGGCAAGGGCAGC CTGAAGGGCCAGCCTGGGGACATCTACCACAAACCTGGGCTAGGTACT TTGTCAAATTCCTGGATGCCATATGCTGAGCATAAGCTGCAATTTTGGGC TGTTACAGCTGAGAATGAGCCTTCTGCAGGCCTGCTGTCTGGATATCCT TTCCAGTGCCCTGGGCTTCACCCCTGAGCACCAGAGAGACTTTATTGCCA GAGATCTGGGGCCAACCCTGGCTAACAGCACACACCACAATGTGAGGCT GCTGATGCTGGATGACCAGAGGCTGCTGCTCCCCCACTGGGCAAGGTG GTGCTGACAGATCCAGAGGCTGCCAAATATGTGCATGGGATTGCTGTCC ACTGGTACCTGGATTTCCTGGCCCCCTGCCAAGGCCACCCTGGGAGAAAC ACATAGACTGTTTCCTAATACCATGCTGTTTGCCTCTGAGGCCTGTGTG GGCAGCAAGTTCTGGGAACAATCTGTGAGACTGGGCAGCTGGGACATCAG GAATGCAGTACAGCCACAGCATCATTACCAACCTGCTGTATCATGTGGT GGGCTGGACAGACTGGAACCTGGCCCTCAACCCTGAAGGGGGCCCCAAC TGGGTGAGGAACCTTGTGGACTCTCCTATCATTGTGGATATTACCAAGG ATACCTTCTACAAGCAGCCTATGTTCTACCACCTGGGCCACTTCAGCAA GTTTCATCCCAGAGGGATCTCAGAGGGTGGGCCTTGTGGCATCTCAGAAA AATGACCTAGATGCTGTTGCCCTGATGCACCCTGATGGGTCAGCAGTGG TAGTGGTCCTGAATAGAAGCTCCAAGGATGTGCCACTGACTATCAAGGA CCCAGCTGTAGGATTTCTGGAACCATCAGTCCTGGGTACAGCATCCAC ACTTACCTCTGGAGGAGGCAG	
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[0237] In some embodiments, a nucleotide sequence encoding a GBA1 protein described herein comprises a reduced number of CpG motifs (e.g., lacking all CpG motifs), e.g., relative to the nucleotide sequence of SEQ ID NO: 1776 or 1777.

[0238] In some embodiments, the encoded GBA1 protein comprises the amino acid sequence of SEQ ID NO: 1774 or SEQ ID NO: 1775.

[0239] In some embodiments, the nucleotide sequence encoding the GBA1 protein or functional variant thereof comprises the nucleotide sequence of SEQ ID NO: 2002. In some embodiments, the nucleotide sequence encoding the GBA1 protein comprises the sequence of SEQ ID NO: 2001, wherein the encoded GBA1 protein comprises a signal sequence, wherein the signal sequence is encoded by the nucleotide sequence of SEQ ID NO: 2005.

[0240] In some embodiments, a codon-optimized nucleotide sequence encoding a GBA1 protein described herein (e.g., SEQ ID NO: 2001 or SEQ ID NO: 2002) replaces a donor splice site, e.g., a nucleotide sequence comprising the sequence of AGGGTAAGC or nucleotides 49 of the 117 numbered according to the nucleotide sequence of SEQ ID NO: 1776, with the nucleotide sequence of AGAGTGTCC, e.g., comprising at least one, two, three, or four modifications, e.g., mutations relative to the nucleotide sequence of AGGGTAAGC, or nucleotides 49 of the 117 numbered according to the nucleotide sequence of SEQ ID NO: 1776. In some embodiments, a codon-optimized nucleotide sequence encoding a GBA1 protein described herein (e.g., SEQ ID NO: 2001 or SEQ ID NO: 2002) contains more than 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140 or more unique modifications, e.g., mutations, compared to the nucleotide sequence of SEQ ID

NO: 1776. In some embodiments, a codon-optimized nucleotide sequence of a GBA1 protein described herein (e.g., SEQ ID NO: 2001 or SEQ ID NO: 2002) comprises a unique GC content profile. Without wishing to be bound by theory, it is believed that, in some embodiments, altering the GC-content of a nucleotide sequence of a GBA1 protein described herein enhances the expression of the codon-optimized nucleotide sequence in a cell (e.g., a human cell or a neuronal cell). In some embodiments, a codon-optimized nucleotide sequence of a GBA1 protein described herein (e.g., SEQ ID NO: 2001 or SEQ ID NO: 2002) has reduced GC-content relative to a wild-type GBA1 nucleotide sequence. In some embodiments, a codon-optimized nucleotide sequence of a GBA1 protein described herein (e.g., SEQ ID NO: 2001 or SEQ ID NO: 2002) comprises a reduced number of CpG motif motifs (e.g., lacking all CpG motifs) as compared to a wildtype GBA1 encoding sequence (e.g., comprising the nucleotide sequence of SEQ ID NO: 1776 or 1777). In some embodiments, a codon-optimized nucleotide sequence of a GBA1 protein described herein (e.g., SEQ ID NO: 2001 or SEQ ID NO: 2002) does not contain a CpG motif. Without wishing to be bound by theory, in some embodiments, sequences with depleted CpG nucleotides may reduce *in vivo* toxicity, e.g., immunogenicity.

[0241] In some embodiments, the viral genome comprises a payload region encoding a GCase protein. The encoded GCase protein may be derived from any species, such as, but not limited to human, non-human primate, or rodent.

[0242] In some embodiments, the viral genome comprises a payload region encoding a human (*Homo sapiens*) GCase protein. In some embodiments, the methods disclosed herein may be used to make the GCase protein.

Payload Component: Enhancement Element

[0243] In some embodiments, a viral genome described herein encoding a GBA1 protein comprises an enhancement element or functional variant thereof. In some embodiments, the encoded enhancement comprises a prosaposin (PSAP) protein, a saposin C (SapC) protein, or functional variant thereof; a cell penetrating peptide (e.g., a ApoEII peptide, a TAT peptide, and/or a ApoB peptide) or functional variant thereof; or a lysosomal targeting signal or functional variant thereof.

[0244] In some embodiments, the viral genome comprises a payload region further encoding a prosaposin (PSAP) protein or a saposin C (SapC) protein or functional variant thereof, e.g., as described herein, e.g., in Table 3A or 3B.

Table 3A. Exemplary PSAP and Saposin Sequences

SEQ ID NO:	Type	Species	Description
1750	Protein	<i>Homo sapiens</i>	Prosaposin isoform A preprotein, NP_002769.1

1751	DNA	<i>Homo sapiens</i>	PSAP transcript variant 1, NM_002778.4
1752	Protein	<i>Homo sapiens</i>	Prosaposin isoform B preprotein, NP_001035930.1
1753	DNA	<i>Homo sapiens</i>	PSAP transcript variant 2, NM_001042465.3
1754	Protein	<i>Homo sapiens</i>	Prosaposin isoform C preprotein, NP_001035931.1
1755	DNA	<i>Homo sapiens</i>	PSAP transcript variant 3, NM_001042466.3
1756	Protein	<i>Homo sapiens</i>	hSapA, amino acids 60 to 140 of SEQ ID NO: 1750: SLPCDICKDVVTAAGDMLKDNATEEEILVYLEKTCDWLPKPNMSASCK EIVDSYLPVILDIIKGEMSRPGEVCSALNLCES
1757	Protein	<i>Homo sapiens</i>	hSapB, amino acids 195 to 275 of SEQ ID NO: 1750: GDVCQDCIQMVTDIQTAVRTNSTFVQALVEHVKEECDRLGPGMADICK NYISQYSEIAIQMMHMQPKEICALVGFCDEVK
1758	Protein	<i>Homo sapiens</i>	hSapC, amino acids 311 to 390 of SEQ ID NO: 1750: SDVYCEVCEFLVKEVTKLIDNNKTEKEILDADFDMCSKLPKSLSEECQ EVVDTYGSSILSILLEEVSPELVCSMLHLCSG
1784	Protein	<i>Homo sapiens</i>	hSapD, amino acids 405 to 486 of SEQ ID NO 1750: DGGFCEVCKKLVGYLDRNLEKNSTKQEI LAALEKGCSFLDPYQKQCDQ FVAEYEPVLIIEILVEVMDPSFVCLKIGACPSAH
1856	DNA	<i>Homo sapiens</i>	Signal Sequence atgtacgccctcttccctcctgccagcctcctggcgcggtctagcc
1857	Protein	<i>Homo sapiens</i>	Signal Sequence MYALFLLASLLGAALA

Table 3B. Exemplary Enhancement Elements

Description	Sequence	SEQ ID NO:
PSAP or Saposin		
PSAP (signal sequence underlined)-nt	atgtacgccctcttccctcctgccagcctcctggcgcggtctagc <u>cgccccgggtccttggactgaaagaatgcaccaggggctcggcagtg</u> ggtgccagaatgtgaagacggcgctccgactgcggggcagtgaaagcac tgctgcagaccgtttggaacaagccaacagtgaatcccttccctg cgacatatgcaaagacgttgtcaccgcagctggtgatatgctgaagg acaatgccactgaggaggagatccttggtttacttggagaagacctgt gactggcttccgaaaccgaacatgtctgcttcattgcaaggagatagt ggactcctacctccctgtcatcctggacatcattaaaggagaaatga gccgtcctggggagggtgtgctctgctctcaacctctgcgagctcttc cagaagcacctagcagagctgaatcaccagaagcagctggagtccaa taagatcccagagctggacatgactgaggtggtggcccccttcatgg ccaacatccctctcctcctcctaccctcaggacggccccgcagcaag ccccagccaaaggataatggggacgtttgccaggactgcattcagat ggtgactgacatccagactgctgtacggaccaactccacctttgtcc aggccttgggtggaacatgtcaaggaggagtgtgaccgcctgggccct ggcatggccgacatatgcaagaactatatcagccagttattctgaaat tgctatccagatgatgatgcacatgcaaccaaggagatctgtgctgc tggttgggttctgtgatgaggtgaaagagatgcccatgcagactctg gtccccgccaaagtggcctccaagaatgtcatccctgccctggaact ggtggagcccatgaagaagcacgaggtcccagcaaagtctgatgttt actgtgaggtgtgtgaattcctgggtgaaggaggtgaccaagctgatt gacaacaacaagactgagaaagaaatactcgacgcttttgacaaaat gtgctcgaagctgccgaagtccctgtcggaagagtgcaggagggtgg tggacacgtacggcagctccatcctgtccatcctgctggaggaggtc agccctgagctggtgtgcagcatgctgcacctctgctctggcagcg gctgcctgcactgaccgttcacgtgactcagccaaaggacggtggct tctgcgaagtgtgcaagaagctgggtgggttatttgatcgcaacctg gagaaaaacagcaccgaagcaggagatcctggctgctcttgagaaagg ctgcagcttccctgccagacccttaccagaagcagtgatgatcagtttg	1858

	tggcagagtagcagagcccggtgctgatcgagatcctggtggaggtgatg gatccttccttcgtgtgcttgaaaattggagcctgccctcggccca taagcccttggtgggaactgagaagtgtatatggggcccaagctact ggtgccagaacacagagacagcagcccagtgcaatgctgtcgagcat tgcaaacgccatgtgtggaactag	
PSAP (no signal sequence)-nt	ggccccggtccttggactgaaagaatgcaccaggggctcggcagtggtg gtgccagaatgtgaagacggcgtccgactgcggggcagtgaaact gcctgcagaccgttttgaacaagccaacagtgaaatcccttccttgc gacatatgcaaagacgttgtcaccgcagctggtgatgctgaagga caatgccactgaggaggagatccttgtttacttggagaagacctgtg actggcttccgaaaccgaacatgtctgtctcatgcaaggagatagtg gactcctacctccctgtcatcctggacatcattaaaggagaaatgag ccgtcctggggaggtgtgctctgctctcaacctctgcgagtgctctcc agaagcacctagcagagctgaatcaccagaagcagctggagtgccaat aagatcccagagctggacatgactgaggtggtggcccccttcatggc caacatccctctcctcctctaccctcaggacggcccccgagcaagc cccagccaaaggataatggggacgtttgccaggactgcattcagatg gtgactgacatccagactgctgtacggaccaactccacctttgtcca ggccttgggtggaacatgtcaaggaggagtggtgaccgctggggcctg gcatggccgacatatgcaagaactatatcagccagtattctgaaatt gctatccagatgatgatgcacatgcaacccaaggagatctgtgcgct ggttgggttctgtgatgaggtgaaagagatgccatgcagactctgg tccccgccaaagtggcctccaagaatgtcatccctgcctggaactg gtggagcccatgaagaagcacgaggtcccagcaaaagtctgatgtta ctgtgaggtgtgtgaattcctggtgaaggagtgaccaagctgattg acaacaacaagactgagaaagaaatactcgacgcttttgacaaaatg tgctcgaagctgccgaagtccctgtcgggaagagtgcaggaggtggt ggacacgtacggcagctccatcctgtccatcctgctggaggaggtca gccctgagctggtgtgcagcatgctgcacctctgctctggcacgcgg ctgcctgcactgaccgttcacgtgactcagccaaaggacggtggctt ctgcgaagtgtgcaagaagctggtgggttatttggatcgcaacctgg agaaaaacagcaccaagcaggagatcctggctgctcttgagaaaggc tgcagcttccctgccagacccttaccagaagcagtgatgagttgtg ggcagagtacgagcccggtgctgatcgagatcctggtggaggtgatgg atccttccttcgtgtgcttgaaaattggagcctgccccctcggccat aagcccttggtgggaactgagaagtgtatatggggcccaagctactg gtgccagaacacagagacagcagcccagtgcaatgctgtcgagcatt gcaaacgccatgtgtggaactag	1859
PSAP (signal sequence underlined)-aa	<u>MYALFLLASLLGAAL</u> AGPVLGLKECTRGS AVWCQNVKTASDCGAVKH CLQTVWNKPTVKSLPCDICKDVVTAAGDMLKDNATEEEIILVYLEKTC DWLPKPNMSASCKEIVDSYLPVILDIKIGEMSRPGEVCSALNLCESL QKHLAELNHQKQLESNKIPELDMTEVVAPFMANIPLLLYPQDGPRSK PQPKDNGDVCQDCIQMVTDIQTAVRTNSTFVQALVEHVKEECDRLGP GMADICKNYISQYSEIAIQMMMHMQPKEICALVGFCEVKEPMQTL VPAKVASKNVI PALELVEPIKKHEVPAKSDVYCEVCEFLVKEVTCLI DNNKTEKEILDAFDKMC SKLPKSLSEECQEVVDTYGSSILSILLEEV SPELVCSMLHLCSGTRLPALTVHVTQPKDGGFCEVCKKLVGYLDRNL EKNSTKQEILAALEKGC SFLPDYQKQCDQFVAEYEPVLI EILVEVM DPSFVCLKIGACPSAHKPLLGTEKCIWGPSYWCQNTETAAQCNAVEH CKRHVWN	1750
PSAP (no signal sequence)	GPVLGLKECTRGS AVWCQNVKTASDCGAVKH CLQTVWNKPTVKSLPC DICKDVVTAAGDMLKDNATEEEIILVYLEKTCDWLPKPNMSASCKEIV DSYLPVILDIKIGEMSRPGEVCSALNLCESLQKHLAELNHQKQLESN KIPELDMTEVVAPFMANIPLLLYPQDGPRSKPQPKDNGDVCQDCIQM VTDIQTAVRTNSTFVQALVEHVKEECDRLGPGMADICKNYISQYSEI	1785

	AIQMMMHMQPKEICALVGFCEVKEMPMQTLVPAKVASKNVIPALEL VEPIKKHEVPAKSDVYCEVCEFLVKEVTKLIDNNKTEKEILDAFDKM CSKLPKSLSEECQEVVDITYGSSILSILLEEVSPELVCSMLHLCSGTR LPALT VHVTQPKDGGFCEVCKKLVGYLDRNLEKNSTKQEI LAALEKG CSFLPDYPYQKQCDQFVAEYEPVLIEILVEVMDPSFVCLKIGACPSAH KPLLGTCKCIWGPSYWCQNTETAAQCNAVEHCKRHVWN	
SAPC (signal sequence underlined)-nt	<u>atgtacgccctcttctcctcctggccagcctcctgggcgcggctctagc</u> <u>cgtgaaagagatgcccatgcagactctggtccccgcaaagtggcct</u> ccaagaatgtcatccctgcccctggaactggtggagcccatgaagaag cacgaggtcccagcaaagtctgatgtttactgtgaggtgtgtgaatt cctggtgaaggaggtgaccaagctgattgacaacaacaagactgaga aagaaatactcgacgcttttgacaaaatgtgctcgaagctgccgaag tcctgtcggaagagtgccaggaggtggtggacacgtacggcagctc catcctgtccatcctgctggaggaggtcagccctgagctggtgtgca gcatgctgcacctctgctctggc	1786
SAPC (no signal sequence)-nt	gtgaaagagatgcccatgcagactctggtccccgcaaagtggcctc caagaatgtcatccctgcccctggaactggtggagcccatgaagaagc acgaggtcccagcaaagtctgatgtttactgtgaggtgtgtgaattc ctggtgaaggaggtgaccaagctgattgacaacaacaagactgagaa agaaatactcgacgcttttgacaaaatgtgctcgaagctgccgaagt ccctgtcggaagagtgccaggaggtggtggacacgtacggcagctcc atcctgtccatcctgctggaggaggtcagccctgagctggtgtgcag catgctgcacctctgctctggc	1787
SAPC (signal sequence underlined)-aa	<u>MYALFLLASLLGAALAVKEMPMQTLVPAKVASKNVIPALELVEPIKK</u> <u>HEVPAKSDVYCEVCEFLVKEVTKLIDNNKTEKEILDAFDKMCSKLPK</u> <u>SLSEECQEVVDITYGSSILSILLEEVSPELVCSMLHLCSG</u>	1788
SAPC (no signal sequence)-aa	VKEMPMQTLVPAKVASKNVIPALELVEPIKKHEVPAKSDVYCEVCE LVKEVTKLIDNNKTEKEILDAFDKMCSKLPKSLSEECQEVVDITYGSS ILSILLEEVSPELVCSMLHLCSG	1789
SAPCv2 (signal sequence underlined)-nt	<u>atgtacgccctcttctcctcctggccagcctcctgggcgcggctctagc</u> <u>ctctgatgtttactgtgaggtgtgtgaattcctggtgaaggaggtga</u> ccaagctgattgacaacaacaagactgagaaagaaatactcgacgct tttgacaaaatgtgctcgaagctgccgaagtccctgtcggaagagtg ccaggaggtggtggacacgtacggcagctccatcctgtccatcctgc tgaggaggtcagccctgagctggtgtgcagcatgctgcacctctgc tctggc	1790
SAPCv2 (no signal sequence)-nt	tctgatgtttactgtgaggtgtgtgaattcctggtgaaggaggtgac caagctgattgacaacaacaagactgagaaagaaatactcgacgctt ttgacaaaatgtgctcgaagctgccgaagtccctgtcggaagagtg caggaggtggtggacacgtacggcagctccatcctgtccatcctgct ggaggaggtcagccctgagctggtgtgcagcatgctgcacctctgct ctggc	1791
SAPCv2 (signal sequence underlined)-aa	<u>MYALFLLASLLGAALASDVYCEVCEFLVKEVTKLIDNNKTEKEILDA</u> <u>FDKMCSKLPKSLSEECQEVVDITYGSSILSILLEEVSPELVCSMLHLC</u> <u>SG</u>	1792
SAPCv2 (no signal sequence) -aa	SDVYCEVCEFLVKEVTKLIDNNKTEKEILDAFDKMCSKLPKSLSEEC QEVVDITYGSSILSILLEEVSPELVCSMLHLCSG	1758
Cell Penetrating Peptides		
TAT – nt	tatggcaggaaaaagcggaggcgaaggcgccgccccccccag	1793
TAT – aa	YGRKKRRQRRRPPQ	1794

ApoB – nt	tccgtaatcgacgccttacagtataagctggaggggaaccaccagatt gacaaggaaacgaggggcttaagcttgctactgcactatccctgagca ataaat	1795
ApoB – aa	SVIDALQYKLEGTTRLTRKRGLKLATALSLSNKF	1796
ApoEII – nt	ctacggaagctgcggaagcggctactgctgcggaacttcggaaacg gctactg	1797
ApoEII – aa	LRKLRKRLLLRKLRKRL	1798
Lysosomal Targeting Sequence (LTS)		
LTS1 – nt	aagtttgaaagacag	1799
LTS1 – aa	KFERQ	1800
LTS2 – nt	atgaaggagaccgctgctgcaaagttcgagagacagcatatggatag ctccacaagcgccgca	1801
LTS2 – aa	MKETAAAKFERQHMDSSSTAA	1802
LTS3 – nt	cagaaaatcctggat	1803
LTS3 – aa	QKILD	1804
LTS4 – nt	cagagattcttcgag	1805
LTS4 – aa	QRFFE	1806
LTS5 – nt	aagtttgaaagacagcagaaaaatcctggatcagagattcttcgag	1807
LTS5 – aa	KFERQQKILDQRFFE	1808

Exemplary GBA1 AAV Viral Genome Sequence Regions and ITR-to-ITR Sequences

[0245] In some embodiments, a viral genome, e.g., an AAV viral genome or vector genome, described herein, comprises a promoter operably linked to a transgene encoding a GBA1 protein. In some embodiments, the viral genome further comprises an inverted terminal repeat region, an enhancer, an intron, a miR binding site, a polyA region, or a combination thereof. Exemplary sequence regions within ITR-to-ITR sequences for viral genomes according to the description are provided in Table 4.

Table 4. Exemplary Viral Genome sequence regions in ITR-to-ITR constructs

Description	Sequence	SEQ ID NO:
ITR (130nt)	ctgcgcgctcgctcgctcactgagggccgcccgggcaaagcccgggctcgggcg acctttggtcgcccgccctcagtgagcgagcgagcgcgagagaggagtgcc aactccatcactaggggttcct	1829
ITR(130nt)	aggaaccctagtgatggagttggccactccctctctgcgcgctcgctcgctca ctgagggccggcgaccaaaggtcgcccgacgcccgggctttgccggggcgccct cagtgagcgagcgagcgcgag	1830
CMVie enhancer	GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTC ATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTG GCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCA TAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT AAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTA TTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCT TATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCA TG	1831

CB promoter	ccacgttctgcttcactctccccatctccccccctccccaccccaattttgt atattttattttttaattattttgtgcagcgatggggcgggggggggcg cgcgccaggcgggcgggcgggcgaggcgggcgggcgaggcgagagg tgcgcgggcagccaatcagagcgggcgctccgaaagtttcttttatggcgag gcgggcgggcgggcgggccctataaaaagcgaagcgcgcgggcg	1834
Human beta globin intron (hGBint)	tcagatcgcttgagacgccatccacgctgttttgacctccatagaagacaccg ggaccgatccagcctccgcggttcgaatcccggccgggaacggtgcattggaa cgcggttccccgtgccaagagtgcgtaagtaccgcctatagagtctataggc ccacaaaaatgctttcttcttttaatactttttgtttatcttatttctaa tactttccctaatactctttctttcagggcaataatgatacaatgtatcatgcct ctttgcaccattctaaagaataacagtgataatttctgggttaaggcaatagca atatttctgcatataaaatatttctgcatataaattgtaactgatgtaagaggtt tcataattgctaatagcagctacaatccagctaccattctgcttttattttatgg ttgggataaggctggattattctgagtcgaagctaggcccttttgcataatcatg ttcatacctcttatcttctccacagctcctgggcaacgtgctggtctgtgtg ctggcccatcactttggcaaagaatt	1842
polyA signal sequence	gatcttttccctctgcaaaaattatggggacatcatgaagcccttgagcat ctgacttctggctaataaaggaaatttattttcattgcaatagtgtgttggaa ttttgtgtctctcactcg	1846
miR183 binding site	AGTGAATTCTACCAGTGCCATA	1847
Spacer	GATAGTTA	
miR183 binding site series	AGTGAATTCTACCAGTGCCATAGATAGTTAAGTGAATTCTACCAGTGCCATAGA TAGTTAAGTGAATTCTACCAGTGCCATAGATAGTTAAGTGAATTCTACCAGTGC CATA	1849
Signal Sequence – nt	ATGGAATTCTCTAGCCCATCTAGAGAGGAATGTCCTAAGCCTCTGTCAAGAGTG TCCATCATGGCCGGCAGCCTGACAGGCCTGCTGCTGCTGCAGGCCGTGTCTTGG GCCAGTGGA	1850
Signal sequence – nt	ATGGAATTCTCTAGCCCATCTAGAGAGGAATGTCCTAAGCCTCTGTCAAGAGTG TCCATCATGGCTGGCAGCCTGACAGGCCTGCTGCTGCTGCAGGCTGTGTCTTGG GCCAGTGGA	2005
Signal Sequence – aa	MEFSSPSREECPKPLSRVSIIMAGSLTGLLLLQAVSWASG	1853

[0246] In some embodiments, the viral genome comprises an inverted terminal repeat sequence region (ITR) provided in Table 4, or a nucleotide sequence with at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% sequence identity to any of the ITR sequences in Table 5.

[0247] This disclosure also provides, in some embodiments, a GBA1 protein encoded by SEQ ID NO 2001 or a nucleotide sequence having at least at least 93%, at least 94%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity thereto or SEQ ID NO 2002 or a nucleotide sequence having at least at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity thereto. In some embodiments, the viral genome comprises a promoter comprising the nucleotide sequence SEQ ID NO 1834 or a nucleotide sequence having at least at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto.

[0248] In some embodiments, the viral genome of an AAV particle described herein comprises the nucleotide sequence, e.g., the nucleotide sequence from the 5' ITR to the 3' ITR, of the nucleotide sequences of SEQ ID NO: 2006 and SEQ ID NO: 2007 or a sequence that is at least 97%, at least 98%, or at least 99% identical thereto.

[0249] This disclosure also provides in some embodiments, a GBA1 protein (e.g., a GBA1 protein) encoded by SEQ ID NO: 2001 or a sequence that is at least 93% identical thereto or SEQ ID NO: 2002 or a sequence that is at least 94% identical thereto.

[0250] In some embodiments, a viral genome encoding a GBA1 protein is a wtGBA1 viral genome, wherein the viral genome comprises a codon-optimized nucleotide sequence encoding a wildtype GBA1 protein, wherein the nucleotide sequence comprises a reduced number of CpG nucleotides (e.g., lacking all CpG motifs), as compared to a wildtype GBA1 encoding sequence (e.g., comprising the nucleotide sequence of SEQ ID NO: 1776 or 1777). In some embodiments, a viral genome encoding a GBA1 protein is a wtGBA1 viral genome, wherein the viral genome comprises a codon-optimized nucleotide sequence encoding a wildtype GBA1 protein, wherein the nucleotide sequence does not comprise any CpG nucleotides.

Table 5. Exemplary Viral Genome (ITR-to-ITR) sequences

Construct ID	Description (5' to 3')	SEQ ID NO:	Length
GBA_VG1	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1759	3413
GBA_VG2	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); Lysosomal targeting sequence 1 (LTS1) (SEQ ID NO: 1799); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1760	3428
GBA_VG3	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); Lysosomal targeting sequence 2 (LTS2) (SEQ ID NO: 1801); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1761	3476
GBA_VG4	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); Lysosomal	1762	3428

	targeting sequence 3 (LTS3) (SEQ ID NO: 1803); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)		
GBA_VG5	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); Lysosomal targeting sequence 4 (LTS4) (SEQ ID NO: 1805); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1763	3428
GBA_VG6	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); G4S3 linker coding sequence (SEQ ID NO: 1730); ApoEII coding sequence (SEQ ID NO: 1797); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1764	3512
GBA_VG7	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); G4S3 linker coding sequence (SEQ ID NO: 1730); TAT coding sequence (SEQ ID NO: 1793); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1765	3500
GBA_VG8	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); signal sequence (SEQ ID NO: 1852); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); Furin cleavage site coding sequence (SEQ ID NO: 1724); T2A coding sequence (SEQ ID NO: 1726); signal sequence (SEQ ID NO: 1856); Prosaposin (PSAP) coding sequence (SEQ ID NO: 1859); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1766	4463
GBA_VG9	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); Furin cleavage site coding sequence (SEQ ID NO: 1724); T2A coding sequence (SEQ ID NO: 1726); signal sequence (SEQ ID NO: 1856); SAPC coding sequence (SEQ ID NO: 1787); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1767	3878
GBA_VG10	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); Furin cleavage site coding sequence (SEQ ID NO: 1724); T2A coding sequence (SEQ ID NO: 1726); signal sequence (SEQ ID NO: 1856); SAPCv2 coding sequence (SEQ ID NO: 1791); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1768	3767
GBA_VG11	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); GBA1	1769	3560

	Variant 3 coding sequence (SEQ ID NO: 1781); G4S3 linker coding sequence (SEQ ID NO: 1730); ApoB coding sequence (SEQ ID NO: 1795); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)		
GBA_VG12	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); TAT coding sequence (SEQ ID NO: 1793); G4S3 linker coding sequence (SEQ ID NO: 1730); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1770	3500
GBA_VG13	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); Lysosomal targeting sequence 5 (LTS5) (SEQ ID NO: 1807); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1771	3458
GBA_VG14	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); Lysosomal targeting sequence 2 (LTS2) (SEQ ID NO: 1801); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); Furin cleavage site coding sequence (SEQ ID NO: 1724); T2A coding sequence (SEQ ID NO: 1726); signal sequence (SEQ ID NO: 1856); SAPC coding sequence (SEQ ID NO: 1787); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1809	3941
GBA_VG15	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); G4S3 linker coding sequence (SEQ ID NO: 1730); ApoEII coding sequence (SEQ ID NO: 1797); Furin cleavage site coding sequence (SEQ ID NO: 1724); T2A coding sequence (SEQ ID NO: 1726); signal sequence (SEQ ID NO: 1856); SAPC coding sequence (SEQ ID NO: 1787); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1810	3977
GBA_VG16	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1852); Lysosomal targeting sequence 2 (LTS2) (SEQ ID NO: 1801); GBA1 Variant 3 coding sequence (SEQ ID NO: 1781); G4S3 linker coding sequence (SEQ ID NO: 1730); ApoEII coding sequence (SEQ ID NO: 1797); Furin cleavage site coding sequence (SEQ ID NO: 1724); T2A coding sequence (SEQ ID NO: 1726); signal sequence (SEQ ID NO: 1856); SAPC coding sequence (SEQ ID NO: 1787); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1811	4040
GBA_VG17	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint)	1812	3413

	(SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1850); GBA1 Variant 1 coding sequence (SEQ ID NO: 1773); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)		
GBA_VG18	ITR (SEQ ID NO: 1829); EF-1 α promoter variant 2 (SEQ ID NO: 1839); signal sequence (SEQ ID NO: 1850); GBA1 Variant 1 coding sequence (SEQ ID NO: 1773); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1813	3375
GBA_VG19	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CMV promoter (SEQ ID NO: 1832); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1850); GBA1 Variant 1 coding sequence (SEQ ID NO: 1773); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1814	3360
GBA_VG20	ITR (SEQ ID NO: 1829); CAG promoter (SEQ ID NO: 1835); signal sequence (SEQ ID NO: 1850); GBA1 Variant 1 coding sequence (SEQ ID NO: 1773); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1815	3901
GBA_VG21	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1851); GBA1 Variant 2 coding sequence (SEQ ID NO: 1777); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1816	3413
GBA_VG22	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1851); GBA1 Variant 2 coding sequence (SEQ ID NO: 1777); Furin cleavage site coding sequence (SEQ ID NO: 1724); T2A coding sequence (SEQ ID NO: 1726); signal sequence (SEQ ID NO: 1856); SAPC coding sequence (SEQ ID NO: 1787); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1817	3878
GBA_VG23	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1851); GBA1 Variant 2 coding sequence (SEQ ID NO: 1777); G4S3 linker coding sequence (SEQ ID NO: 1730); ApoEII coding sequence (SEQ ID NO: 1797); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1818	3512
GBA_VG24	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1851); Lysosomal targeting sequence 2 (LTS2) (SEQ ID NO: 1801); GBA1 Variant 2 coding sequence (SEQ ID NO: 1777); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1819	3476
GBA_VG25	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1851); GBA1 Variant 2 coding sequence (SEQ ID NO: 1777); Lysosomal	1820	3428

	targeting sequence 4 (LTS4) (SEQ ID NO: 1805); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)		
GBA_VG26	ITR (SEQ ID NO: 1829); EF -1 α promoter variant 3 (SEQ ID NO: 1840); signal sequence (SEQ ID NO: 1851); GBA1 Variant 2 coding sequence (SEQ ID NO: 1777); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1821	3375
GBA_VG27	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1850); GBA1 Variant 1 coding sequence (SEQ ID NO: 1773); Furin cleavage site coding sequence (SEQ ID NO: 1724); T2A coding sequence (SEQ ID NO: 1726); signal sequence (SEQ ID NO: 1856); SAPC coding sequence (SEQ ID NO: 1787); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1822	3878
GBA_VG28	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1850); GBA1 Variant 1 coding sequence (SEQ ID NO: 1773); G4S3 linker coding sequence (SEQ ID NO: 1730); ApoEII coding sequence (SEQ ID NO: 1797); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1823	3512
GBA_VG29	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1850); Lysosomal targeting sequence 2 (LTS2) (SEQ ID NO: 1801); GBA1 Variant 1 coding sequence (SEQ ID NO: 1773); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1824	3476
GBA_VG30	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1850); GBA1 Variant 1 coding sequence (SEQ ID NO: 1773); Lysosomal targeting sequence 4 (LTS4) (SEQ ID NO: 1805); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1825	3428
GBA_VG31	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1851); GBA1 Variant 2 coding sequence (SEQ ID NO: 1777); G4S3 linker coding sequence (SEQ ID NO: 1730); TAT coding sequence (SEQ ID NO: 1793); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1826	3500
GBA_VG32	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1850); GBA1 Variant 1 coding sequence (SEQ ID NO: 1773); G4S3 linker coding sequence (SEQ ID NO: 1730); TAT coding sequence (SEQ ID NO: 1793); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1827	3500

GBA_VG33	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1850); GBA1 Variant 1 coding sequence (SEQ ID NO: 1773); miR183 binding site (SEQ ID NO: 1847); Spacer (SEQ ID NO: 1848); miR183 binding site (SEQ ID NO: 1847); Spacer (SEQ ID NO: 1848); miR183 binding site (SEQ ID NO: 1847); Spacer (SEQ ID NO: 1848); mir183 binding site (SEQ ID NO: 1847); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1828	3571
GBA_VG34	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 1851); GBA1 Variant 2 coding sequence (SEQ ID NO: 1777); miR183 binding site (SEQ ID NO: 1847); Spacer (SEQ ID NO: 1848); miR183 binding site (SEQ ID NO: 1847); Spacer (SEQ ID NO: 1848); miR183 binding site (SEQ ID NO: 1847); Spacer (SEQ ID NO: 1848); mir183 binding site (SEQ ID NO: 1847); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	1870	3571
GBA1_VG35	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 2005); GBA1 Variant 4 coding sequence (SEQ ID NO: 2002); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	2006	3413
GBA_VG36	ITR (SEQ ID NO: 1829); CMVie (SEQ ID NO: 1831); CB promoter (SEQ ID NO: 1834); human beta globin intron (hGBint) (SEQ ID NO: 1842); signal sequence (SEQ ID NO: 2005); GBA1 Variant 4 coding sequence (SEQ ID NO: 2002); miR183 binding site (SEQ ID NO: 1847); Spacer (SEQ ID NO: 1848); miR183 binding site (SEQ ID NO: 1847); Spacer (SEQ ID NO: 1848); miR183 binding site (SEQ ID NO: 1847); Spacer (SEQ ID NO: 1848); miR183 binding site (SEQ ID NO: 1847); polyA signal region (SEQ ID NO: 1846); ITR (SEQ ID NO: 1830)	2007	3571

Table 6. Exemplary ITR-to-ITR sequences encoding a GBA1 protein

Construct ID	Sequence	SEQ ID NO:
GBA_VG17	ctgcgcgctcgctcgctcactgagggccgcccgggcaaagcccgggcgctcgggcgac ctttggtcgcccggcctcagtgagcgagcgagcgcgagagagggagtgcccaact ccatcactaggggttccttgtagttaatgattaaccgcgatgctacttatctacc agggtaatggggatcctctagaactatagctagtcGACATTGATTATTGACTAGTT ATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCG GTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCC ATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATT GACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTG TATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTG GCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACG TATTAGTCATCGCTATTACCATGtcgagggccacgttctgcttcactctccccatct ccccccctccccacccccaattttgtatttatttattttttaattatttgtgca gcatggggcgggggggggggcgcgccagggcgggcgggggcgggcgagggg cgggcgggcgagggcgagaggtgctggcgccagccaatcagagcgggcgcgctccg	1812

	aaagtttccttttatggcgagcgcgcgcgcgcgccctataaaaaagcgaagcg cgcgcgcgcgaggagcaagcttcgttttagtgaaccgtcagatcgccctggagacgcc atccacgctgttttgacctccatagaagacacccgggacccgatccagcctccgcgga ttcgaatcccggcgcggaacggtgcattggaacgcggattccccgtgccaagagt acgtaagtaccgcctatagagctctataggcccaaaaaatgctttcttcttttaa tatactttttgtttatcttatttctaatactttccctaatactctttctttcaggg caataatgatacaatgtatcatgcctctttgcaccattctaaagaataacagtgat aatttctgggttaaggcaatagcaatatttctgcatataaatatttctgcatataa attgtaactgatgtaagaggtttcatattgctaatagcagctacaatccagctacc attctgcttttattttatgggtgggataaggctggattattctgagtcgaagctag gccccttttgctaatacatgttcataacctcttatcttccctcccacagctcctgggcaa cgtgctggtctgtgtgctggcccatcactttggcaaagaattgggatttcgaaccgg tgccgccaccATGGAATTCTCTAGCCCATCTAGAGAGGAATGTCCTAAGCCTCTGT CAAGAGTGTCCATCATGGCCGGCAGCCTGACAGGCCTGCTGCTGCTGCAGGCCGTG TCCTGGGCCAGTGGAGCCCGGCCCTGCATCCCTAAGTCCTTCGGCTATTCTAGCGT GGTCTGCGTGTGTAATGCCACTTACTGCGACAGCTTCGACCCTCCTACCTTCCCCG CCCTTGGAACATTCAGCAGATACGAGAGCACCAGAAGCGGCAGAAGAATGGAACCTG AGCATGGGCCCAATCCAGGCCAACACACCCGGCACCCGGCCTGCTGCTGACACTGCA ACCTGAGCAGAAGTTCAGAAAGGTGAAGGGATTGAGAGCGCCATGACCGACGCTG CTGCTCTGAACATCCTGGCCCTCTCCCCACCTGCTCAGAACCTGCTGCTTAAAAGC TACTTCAGCGAGGAAGGCATCGGCTATAACATCATCAGAGTGCCCATGGCCAGCTG CGACTTCAGCATCAGAACATACACCTACGCCGATACACCTGATGACTTCCAACCTG ACAACCTTCAGCCTGCCTGAAGAGGACACAAAGCTGAAAATCCCCCTGATCCACCGG GCCCTGCAGCTGGCCCAGAGACCTGTGAGCCTGCTGGCCTCTCCTTGACAAAGCCC CACCTGGCTGAAGACCAATGGAGCTGTGAACGGCAAGGGCAGCCTGAAGGGCCAGC CCGGCGACATCTACCACCAACCTGGGCTCGCTACTTCGTGAAATTCTTGACGCC TACGCTGAGCATAAGCTGCAATTTTGGGCCGTTACAGCCGAGAACGAGCCTTCTGC CGGCCTGCTGTCTGGATATCCTTTCCAGTGCCTGGGCTTCACCCCTGAGCACCAGA GAGACTTTATCGCCAGAGATCTGGGGCCTACCCTGGCTAACAGCACACACCACAAC GTGCGGCTGCTGATGCTGGACGATCAGAGGCTGCTGCTCCCCACTGGGCCAAGGT GGTGCTGACAGATCCGGAGGCCGCCAAATACGTGCACGGCATCGCCGTCCACTGGT ACCTGGATTTCCTGGCCCCTGCCAAGGCCACCCTGGGCGAGACACATAGACTGTTT CCTAATACCATGCTGTTTCGCCAGCGAGGCCTGCGTGGGCAGCAAGTTCTGGGAACA GAGCGTGCGGCTGGGCAGCTGGGACAGAGGAATGCAGTACAGCCACAGCATCATTA CCAACCTGCTGTACCACGTGGTGGGCTGGACCGACTGGAACCTGGCCCTGAACCCC GAAGGCGGCCCAACTGGGTGCGGAACTTCGTGGACTCTCCTATCATCGTGGATAT TACCAAGGATACCTTTTACAAGCAGCCTATGTTCTACCACCTGGGCCACTTCAGCA AGTTCATCCCTGAGGGCTCTCAGCGGGTGGGCCTGGTGGCCTCTCAGAAAAACGAC CTGGATGCCGTTGCCCTGATGCACCCGACGGCAGCGCCGTGGTGGTCTGCTCTGAA TAGAAGCTCCAAGGACGTGCCTCTGACCATCAAGGACCCCGCTGTGGGATTTCTGG AAACCATCAGCCCTGGCTACAGCATCCACACCTACCTGTGGCGGCGGCAGtagtaa ctcgaggacggggtgaactacgcctgaggatccgatctttttccctctgccaaaaa ttatggggacatcatgaagcccttgagcatctgacttctggctaataaaggaaat ttattttcattgcaatagtggttggaattttttgtgtctctcactcggcctaggt agataagtagcatggcggttaatcattaactacaaggaacccctagtgatggagt tgccactccctctctgcgcgtcgctcgctcactgaggccgggagcacaaggtc gcccagcggcggtttgcccggcgccctcagtgagcgagcgagcgcgag	
GBA_VG18	ctgcgcgctcgctcgctcactgaggccgcccgggcaaaagccgggctcgggcgac ctttggctgccccggcctcagtgagcgagcgagcgcgagagagggagtgggccaact ccatcactaggggttccttgtagttaatgattaacccgccatgctacttatctacc agggtaatggggatcctctagaactatagctagtcgacataacgcgtgcatgcgtg aggctccggtgcccgtcagtgggcagagcgcacatcgccacagtcccccgagaagt tggggggaggggtcggaattgaaccggtgcctagagaaggtggcgcggggtaaac tgggaaagtgatgtcgtgtactggctccgcctttttcccgaggggtgggggagaacc gtatataagtgcagtagtcgccgtgaacggttctttttcgcaacgggtttgcccga gaacacaggtaagtgcggtgtgtggttcccggggcctggcctctttacgggttat	1813

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	CCGGCGACATCTACCACCAAACCTGGGCTCGCTACTTCGTGAAATTCCTGGACGCC TACGCTGAGCATAAGCTGCAATTTTGGGCCGTTACAGCCGAGAACGAGCCTTCTGC CGGCCTGCTGTCTGGATATCCTTTCCAGTGCCTGGGCTTCACCCCTGAGCACCAGA GAGACTTTATCGCCAGAGATCTGGGGCCTACCCTGGCTAACAGCACACACCACAAC GTGCGGCTGCTGATGCTGGACGATCAGAGGCTGCTGCTCCCCACTGGGCCAAGGT GGTGCTGACAGATCCGGAGGCCGCCAAATACGTGCACGGCATCGCCGTCCACTGGT ACCTGGATTTCTGGCCCCCTGCCAAGGCCACCCTGGGCGAGACACATAGACTGTTT CCTAATACCATGCTGTTCCGCCAGCGAGGCCTGCGTGGGCAGCAAGTTCTGGGAACA GAGCGTGCGGCTGGGCAGCTGGGACAGAGGAATGCAGTACAGCCACAGCATCATT CCAACCTGCTGTACCACGTGGTGGGCTGGACCGACTGGAACCTGGCCCTGAACCCC GAAGGCGGCCCAACTGGGTGCGGAACCTTCGTGGACTCTCCTATCATCGTGGATAT TACCAAGGATACCTTTTACAAGCAGCCTATGTTCTACCACCTGGGCCACTTCAGCA AGTTCATCCCTGAGGGCTCTCAGCGGGTGGGCCTGGTGGCCTCTCAGAAAAACGAC CTGGATGCCGTTGCCCTGATGCACCCCGACGGCAGCGCCGTGGTGGTCTCCTGAA TAGAAGCTCCAAGGACGTGCCTCTGACCATCAAGGACCCCGCTGTGGGATTTCTGG AAACCATCAGCCCTGGCTACAGCATCCACACCTACCTGTGGCGGCGGCAGGGAGGG GGGGGTTTCGGGTGGCGGCGGAAGTGGGGCGGTGGTTCtatggcaggaaaaagcg gaggcaaaggcgccgccccccccagTAGTAACTCGAGGACGGGGTGAACCTACGCCT GAGGATCCgatctttttccctctgcaaaaaattatggggacatcatgaagcccctt gagcatctgacttctggctaataaaggaaatttattttatttgcaatagtgtgttg gaattttttgtgtctctcactcgGCCTAGGTAGATAAGTAGCATGGCGGGTTAATC ATTAACTACAaggaacccttagtgatggagttggccactccctctctgcgcgctcg ctcgctcactgaggccggcgcaaaaggtcgcccgaagcccgggccttggccggg cggcctcagtgagcgagcgagcgcgag	
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	ACCTGAGCAGAAGTTCCAGAAGGTGAAGGGATTTGGAGGCGCCATGACCGACGCTG CTGCTCTGAACATCCTGGCCCTCTCCCCACCTGCTCAGAACCTGCTGCTTAAAAGC TACTTCAGCGAGGAAGGCATCGGCTATAACATCATCAGAGTGCCCATGGCCAGCTG CGACTTCAGCATCAGAACATACACCTACGCCGATACACCTGATGACTTCCAACCTGC ACAACCTTCAGCCTGCCTGAAGAGGACACAAAGCTGAAAATCCCCCTGATCCACCGG GCCCTGCAGCTGGCCCAGAGACCTGTGAGCCTGCTGGCCTCTCCTTGGACAAGCCC CACCTGGCTGAAGACCAATGGAGCTGTGAACGGCAAGGGCAGCCTGAAGGGCCAGC CCGGCGACATCTACCACCAAACCTGGGCTCGCTACTTCGTGAAATTCCTGGACGCC TACGCTGAGCATAAGCTGCAATTTTGGGCCGTTACAGCCGAGAACGAGCCTTCTGC CGGCCTGCTGTCTGGATATCCTTTCAGTGCCTGGGCTTCACCCCTGAGCACCAGA GAGACTTTATCGCCAGAGATCTGGGGCCTACCCTGGCTAACAGCACACACCACAAC GTGCGGCTGCTGATGCTGGACGATCAGAGGCTGCTGCTCCCCACTGGGCCAAGGT GGTGCTGACAGATCCGGAGGCCGCCAAATACGTGCACGGCATCGCCGTCCACTGGT ACCTGGATTTCTGGCCCCTGCCAAGGCCACCCTGGGCGAGACACATAGACTGTTT CCTAATACCATGCTGTTCCGCCAGCGAGGCCTGCGTGGGCGAGCAAGTTCTGGGAACA GAGCGTGCGGCTGGGCAGCTGGGACAGAGGAATGCAGTACAGCCACAGCATCATT CCAACCTGCTGTACCACGTGGTGGGCTGGACCGACTGGAACCTGGCCCTGAACCCC GAAGGCGGCCCAACTGGGTGCGGAACTTCGTGGACTCTCCTATCATCGTGGATAT TACCAAGGATACCTTTTACAAGCAGCCTATGTTCTACCACCTGGGCCACTTCAGCA AGTTTCATCCCTGAGGGCTCTCAGCGGGTGGGCCTGGTGGCCTCTCAGAAAAACGAC CTGGATGCCGTTGCCCTGATGCACCCCGACGGCAGCGCCGTGGTGGTCTGCTCTGAA TAGAAGCTCCAAGGACGTGCCTCTGACCATCAAGGACCCCGCTGTGGGATTTCTGG AAACCATCAGCCCTGGCTACAGCATCCACACCTACCTGTGGCGGCGGCAGTAGTAA CCTCGAGGTACCAGGAGCTCTTCTCCTAGTGAATTCTACCAGTGCCATAGATAGTT AAGTGAATTCTACCAGTGCCATAGATAGTTAAGTGAATTCTACCAGTGCCATAGAT AGTTAAGTGAATTCTACCAGTGCCATACTGCAGTCAGGTCTATACCATCGAGGACG GGGTGAACTACGCCTGAGGATCCgatctttttccctctgccccaaattatggggac atcatgaagccccttgagcatctgacttctggctaataaaggaaatttattttcat tgcaatagtgtgttggaatttttgtgtctctcactcgGCCTAGGTAGATAAGTAG CATGGCGGGTTAATCATTAACATAaggaacccctagtgtatggagttggccactcc ctctctgcgcgctcgctcgctcactgaggccgggagccaaagggtcgcccgagcc cgggctttgccccgggcggcctcagtgagcgagcgagcgcgagcag	
GBA_VG35	ctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgctcgggcgac ctttggctcgcccggcctcagtgagcgagcgagcgcgagagagggagtgggcaact ccatcactaggggttccttgtagttaatgattaaccgcccagtgctacttatctacc agggtaatggggatcctctagaactatagctagtcGACATTGATTATTGACTAGTT ATTAATAGTAATCAATTACGGGGTCATTAGTTTCATAGCCCATATATGGAGTTCCGC GTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCC ATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATT GACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTG TATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTG GCATTATGCCAGTACATGACCTTATGGGACTTTTCTACTTGGCAGTACATCTACG TATTAGTCATCGCTATTACCATGtcgaggccacgttctgcttctactctccccatct ccccccctccccaccccccaattttgtatttatttattttttaattattttgtgca cgcatggggcgggggggggggcgcgcgccaggcgggggcggggagggcgagggg cggggagggcgagggcgagaggtgcggcgccagccaatcagagcgggcgcgctccg aaagtctccttttatggcgagggcgggcgggcgggcgccctataaaaaagcgaagcg cgcggggggagcaagcttcgttttagtgaaccgtcagatcgccctggagagcc atccacgtgttttgacctccatagaagacacccgggaccgatccagcctccgcgga ttcgaatccggcgccggaacggtgcattggaacgaggattccccgtgccaagagt acgtaagtaccgcctatagagtctatagggcccaaaaaatgctttcttcttttaa tatactttttgtttatcttatttctaatactttccctaatactcttttctttcagg caataatgatacaatgtatcatgcctctttgcaccattctaaagaataacagtgtat aatttctgggttaaggcaatagcaatatttctgcatataaatatttctgcatataa attgtaactgatgtaagaggtttcatattgctaatagcagctacaatccagctacc attctgcttttattttatggttgggataaggctggattattctgagtcgaagctag	2006

	gccccttttgctaatacatgttcatacctcttatcttctccacagctcctgggcaa cgtgctggtctgtgtgctggcccatcactttggcaaagaattgggattcgaaccgg tgccgccaccATGGAATTCTCTAGCCCATCTAGAGAGGAATGTCCTAAGCCTCTGT CAAGAGTGTCCATCATGGCTGGCAGCCTGACAGGCCTGCTGCTGCTGCAGGCTGTG TCCTGGGCCAGTGGAGCCAGGCCCTGCATCCCTAAGTCCTTTGGCTATTCTTCTGT GGTCTGTGTGTGTAATGCCACTTACTGTGACAGCTTTGACCCTCCTACCTTCCCTG CCCTTGGAACATTACAGCAGATATGAGAGCACCAGATCTGGCAGAAGAATGGAACCTG AGCATGGGCCCAATCCAGGCCAACACACAGGCACAGGCCTCCTGCTGACACTGCA ACCTGAGCAGAAGTTCAGAAAGTGAAGGGATTGGAGGTGCCATGACAGATGCTG CTGCTCTGAACATCCTGGCCCTCTCCCCACCTGCTCAGAACCTGCTGCTTAAAAGC TACTTCTCTGAGGAAGGCATTGGCTATAACATCATCAGAGTGCCCATGGCCAGCTG TGACTTCAGCATCAGAACATACACTTATGCTGATACACCTGATGACTTCCAACCTGC ACAACTTCAGTCTGCCTGAAGAGGACACAAAGCTGAAAATCCCCCTGATCCACAGG GCCCTCCAGCTGGCCCAGAGACCTGTGAGCCTGCTGGCCTCTCCTTGACAAAGCCC CACCTGGCTGAAGACTAATGGAGCTGTGAATGGCAAGGGCAGCCTGAAGGGCCAGC CTGGGGACATCTACCACCAAACCTGGGCTAGGTACTTTGTCAAATTCCTGGATGCC TATGCTGAGCATAAGCTGCAATTTTGGGCTGTTACAGCTGAGAATGAGCCTTCTGC AGGCCTGCTGTCTGGATATCCTTTCCAGTGCCTGGGCTTCACCCCTGAGCACCAGA GAGACTTTATTGCCAGAGATCTGGGGCCAACCCTGGCTAACAGCACACACCACAAT GTGAGGCTGCTGATGCTGGATGACCAGAGGCTGCTGCTCCCCCACTGGGCCAAGGT GGTGTGACAGATCCAGAGGCTGCCAAATATGTGCATGGGATTGCTGTCCACTGGT ACCTGGATTTCTGGCCCTGCCAAGGCCACCCTGGGAGAAACACATAGACTGTTTT CCTAATACCATGCTGTTTTGCCCTCTGAGGCCTGTGTGGGCAGCAAGTTCTGGGAACA ATCTGTGAGACTGGGCAGCTGGGACAGAGGAATGCAGTACAGCCACAGCATCATTA CCAACCTGCTGTATCATGTGGTGGGCTGGACAGACTGGAACCTGGCCCTCAACCCT GAAGGGGGCCCCAACTGGGTGAGGAACCTTTGTGGACTCTCCTATCATTGTGGATAT TACCAAGGATACCTTCTACAAGCAGCCTATGTTCTACCACCTGGGCCACTTCAGCA AGTTCATCCCAGAGGGATCTCAGAGGGTGGGCCTTGTGGCATCTCAGAAAAATGAC CTAGATGCTGTTGCCCTGATGCACCCTGATGGGTGAGCAGTGGTAGTGGTCTGAA TAGAAGCTCCAAGGATGTGCCACTGACTATCAAGGACCCAGCTGTAGGATTTCTGG AAACCATCAGTCTGGGTACAGCATCCACACTTACCTCTGGAGGAGGCAGtagtaa ctcgaggacggggtgaactacgcctgaggatccgatctttttccctctgccaaaaa ttatggggacatcatgaagcccttgagcatctgacttctggctaataaaggaaat ttattttcattgcaatagtgtgttgaattttttgtgtctctcactcggcctaggt agataagtagcatggcggttaatcattaactacaaggaaccctagtgatggagt tgccactccctctctgcgcgctcgctcgctcactgaggcgggcgaccaaaggtc gcccgcgccccgggctttgcccgggcggcctcagtgagcgagcgagcgcgag	
GBA_VG36	ctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgctcgggcgac ctttggtcgcccggcctcagtgcgagcgagcgcgagagagggagtgggccaact ccatcactaggggttcctTGTAGTTAATGATTAAACCGCCATGCTACTTATCTACC AGGGTAATGGGGATCCTCTAGAACTATAGCTAGTCGACATTGATTATTGACTAGTT ATTAATAGTAATCAATTACGGGGTCATTAGTTTCATAGCCCATATATGGAGTTCGCG GTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCGCCC ATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATT GACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTG TATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTG GCATTATGCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACG TATTAGTCATCGCTATTACCATGTCGAGGccacgttctgttctcactctccccatct ccccccctccccaccccccaattttgtatttatttattttttaattattttgtgca gcgatggggcgggggggggggcgcgcgccaggcgggcgggggcgggcgagggg cgggcgggcgagggcgagaggtgcgcgccagccaatcagagcgggcgcgctccg aaagtttccttttatggcgagcgcgcgggcgggcgccctataaaaagcgaagcg cgcgggcgggCGGGAGCAAGCTTCGTTTAGTGAACCGtcagatcgccctggagacgcc atccacgctgttttgacctccatagaagacacccgggacccgatccagcctccgcgga ttcgaatcccggccgggaacgggtgcattggaacgcggattccccgtgccaaagagt acgtaagtaccgcctatagagtcctataggcccaaaaaatgctttcttcttttaa	2007

	tatacttttttgtttatcttattttctaatactttccctaatactcttttctttcaggg caataatgataacaatgtatcatgcctctttgcaccattctaaagaataacagtgat aatttctgggttaaggcaatagcaatatttctgcatataaatatttctgcatataa attgtaactgatgtaagaggtttcatattgctaatagcagctacaatccagctacc attctgcttttattttatggttgggataaggctggattattctgagtcgaagctag gcccttttgctaatacatgttcataacctcttatcttcctcccacagctcctgggcaa cgtgctggtctgtgtgctggcccatcactttggcaaagaattGGGATTCTGAACCGG TGCCGCCACCATGGAATTCTCTAGCCCATCTAGAGAGGAATGTCTAAGCCTCTGT CAAGAGTGTCCATCATGGCTGGCAGCCTGACAGGCCTGCTGCTGCTGCAGGCTGTG TCCTGGGCCAGTGGAGCCAGGCCCTGCATCCCTAAGTCCTTTGGCTATTCTTCTGT GGTCTGTGTGTGTAATGCCACTTACTGTGACAGCTTTGACCCCTCTACCTTCCCTG CCCTTGGAACATTTCAGCAGATATGAGAGCACCAGATCTGGCAGAAGAATGGAAGT AGCATGGGCCCAATCCAGGCCAACACACAGGCACAGGCCTCCTGCTGACACTGCA ACCTGAGCAGAAGTTCAGAAAGGTGAAGGGATTTGGAGGTGCCATGACAGATGCTG CTGCTCTGAACATCCTGGCCCTCTCCCCACCTGCTCAGAACCTGCTGCTTAAAAGC TACTTCTCTGAGGAAGGCATTGGCTATAACATCATCAGAGTGCCCATGGCCAGCTG TGACTTCAGCATCAGAACATACACTTATGCTGATACACCTGATGACTTCCAACCTGC ACAACCTTCAGTCTGCCTGAAGAGGACACAAAGCTGAAAATCCCCCTGATCCACAGG GCCCTCCAGCTGGCCCAGAGACCTGTGAGCCTGCTGGCCTCTCCTTGACAAAGCCC CACCTGGCTGAAGACTAATGGAGCTGTGAATGGCAAGGGCAGCCTGAAGGGCCAGC CTGGGGACATCTACCACCAACCTGGGCTAGGTACTTTGTCAAATTCCTGGATGCC TATGCTGAGCATAAGCTGCAATTTTGGGCTGTTACAGCTGAGAATGAGCCTTCTGC AGGCCTGCTGTCTGGATATCCTTTCCAGTGCCTGGGCTTCACCCCTGAGCACCAGA GAGACTTTATTGCCAGAGATCTGGGGCCAACCTGGCTAACAGCACACACCACAAT GTGAGGCTGCTGATGCTGGATGACCAGAGGCTGCTGCTCCCCCACTGGGCCAAGGT GGTGCTGACAGATCCAGAGGCTGCCAAATATGTGCATGGGATTGCTGTCCACTGGT ACCTGGATTTCTGGCCCTGCCAAGGCCACCCTGGGAGAAACACATAGACTGTTT CCTAATACCATGCTGTTTGCCCTCTGAGGCCTGTGTGGGCAGCAAGTTCTGGGAACA ATCTGTGAGACTGGGCAGCTGGGACAGAGGAATGCAGTACAGCCACAGCATCATTA CCAACCTGCTGTATCATGTGGTGGGCTGGACAGACTGGAACCTGGCCCTCAACCT GAAGGGGGCCCCAACCTGGGTGAGGAACCTTGTGGACTCTCCTATCATTGTGGATAT TACCAAGGATACCTTCTACAAGCAGCCTATGTTCTACCACCTGGGCCACTTCAGCA AGTTCATCCCAGAGGGATCTCAGAGGGTGGGCCTTGTGGCATCTCAGAAAAATGAC CTAGATGCTGTTGCCCTGATGCACCCTGATGGGTGAGCAGTGGTAGTGGTCCTGAA TAGAAGCTCCAAGGATGTGCCACTGACTATCAAGGACCCAGCTGTAGGATTTCTGG AAACCATCAGTCTGGGTACAGCATCCACACTTACCTCTGGAGGAGGCAGTAGTAA CCTCGAGGTACCAGGAGCTCTTCTCCTAGTGAATTCTACCAGTGCCATAGATAGTT AAGTGAATTCTACCAGTGCCATAGATAGTTAAGTGAATTCTACCAGTGCCATAGAT AGTTAAGTGAATTCTACCAGTGCCATACTGCAGTCAGGTCTATACCATCGAGGACG GGGTGAACTACGCCTGAGGATCCgatctttttccctctgccccaaattatggggac atcatgaagccccttgagcatctgacttctggctaataaaggaaatttattttcat tgcaatagtgtgttggaatttttgtgtctctcactcgGCCTAGGTAGATAAGTAG CATGGCGGGTTAATCATTAACACAaggaacccctagtgatggagtggccactcc ctctctgcgcgctcgctcgctcactgaggccgggacgacaaaggtcgcccagcgcc cgggctttgcccgggcggcctcagtgagcgagcgagcgcgag	
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[0251] In some embodiments, the viral genome of an AAV particle described herein comprises a nucleotide sequence comprising one or more, e.g., all of, the components provided in Table 9 or Table 10, or sequences having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% sequence identity thereto.

[0252] In some embodiments, the viral genome of an AAV particle described herein comprises a GBA1 variant nucleotide sequence comprising SEQ ID NO: 2002, e.g., as shown in Table 9, or a sequence having at least 95% identity thereto. In some embodiments, said AAV particle comprises improved GC content and reduced immunogenicity compared to an AAV particle comprising a GBA1 variant nucleotide sequence comprising SEQ ID NO: 1773, e.g., as shown in Table 7. In some embodiments, the viral genome of an AAV particle described herein comprises a signal sequence and GBA1 variant nucleotide sequence comprising SEQ ID NOs: 2005 and 2002, e.g., as shown in Table 9, or sequences having at least 95% identity thereto. In some embodiments, said AAV particle comprises improved GC content and reduced immunogenicity compared to an AAV particle comprising a signal sequence and GBA1 variant nucleotide sequence comprising SEQ ID NOs: 1850 and 1773, e.g., as shown in Table 7. In some embodiments, the viral genome of an AAV particle described herein comprises a nucleotide sequence comprising SEQ ID NO: 2006, e.g., as shown in Table 9, or a sequence having at least 95% identity thereto. In some embodiments, said AAV particle comprises improved GC content and reduced immunogenicity compared to an AAV particle comprising a nucleotide sequence comprising SEQ ID NO: 1812, e.g., as shown in Table 7.

[0253] In some embodiments, the viral genome of an AAV particle described herein comprises a GBA1 variant nucleotide sequence comprising SEQ ID NO: 2002, e.g., as shown in Table 10, or a sequence having at least 95% identity thereto. In some embodiments, said AAV particle comprises improved GC content and reduced immunogenicity compared to an AAV particle comprising a GBA1 variant nucleotide sequence comprising SEQ ID NO: 1773, e.g., as shown in Table 8. In some embodiments, the viral genome of an AAV particle described herein comprises a signal sequence and GBA1 variant nucleotide sequence comprising SEQ ID NOs: 2005 and 2002, e.g., as shown in Table 10, or sequences having at least 95% identity thereto. In some embodiments, said AAV particle comprises improved GC content and reduced immunogenicity compared to an AAV particle comprising a signal sequence and GBA1 variant nucleotide sequence comprising SEQ ID NOs: 1850 and 1773, e.g., as shown in Table 8. In some embodiments, the viral genome of an AAV particle described herein comprises a nucleotide sequence comprising SEQ ID NO: 2007, e.g., as shown in Table 10, or a sequence having at least 95% identity thereto. In some embodiments, said AAV particle comprises improved GC content and reduced immunogenicity compared to an AAV particle comprising a nucleotide sequence comprising SEQ ID NO: 1828, e.g., as shown in Table 8.

Table 7. Sequence Regions in ITR-to-ITR Sequences

Sequence Regions	GBA_VG17 (SEQ ID NO: 1812)		
	Region SEQ ID NO	Region length	Position in SEQ ID NO: 1812
5' ITR	1829	130	1-130
CMVie	1831	380	204-583
CB promoter	1834	260	590-849
Intron	1842	566	877-1442
Signal sequence	1850	117	1467-1583
GBA1 Variant 1 coding sequence	1773	1,491	1584-3074
PolyA	1846	127	3114-3240
3' ITR	1830	130	3284-3413

Table 8. Sequence Regions in ITR-to-ITR Sequences

Sequence Regions	GBA_VG33 (SEQ ID NO: 1828)		
	Region SEQ ID NO	Region length	Position in SEQ ID NO: 1828
5' ITR	1829	130	1-130
CMVie	1831	380	204-583
CB promoter	1834	260	590-849
Intron	1842	566	877-1442
Signal sequence	1850	117	1467-1583
GBA1 Variant 1 coding sequence	1773	1,491	1584-3074
miR183 binding site	1847	22	3108-3129
Spacer	1848	8	3130-3137
miR183 binding site	1847	22	3138-3159
Spacer	1848	8	3160-3167
miR183 binding site	1847	22	3168-3189
Spacer	1848	8	3190-3197
miR183 binding site	1847	22	3198-3219
PolyA	1846	127	3272-3398
3' ITR	1830	130	3442-3751

Table 9. Sequence Regions in ITR-to-ITR Sequences

Sequence Regions	GBA_VG35 (SEQ ID NO: 2006)		
	Region SEQ ID NO	Region length	Position in SEQ ID NO: 2006
5' ITR	1829	130	1-130
CMVie	1831	380	204-583
CB promoter	1834	260	590-849
Intron	1842	566	877-1442
Signal sequence	2005	117	1467-1583
GBA1 Variant 4 coding sequence	2002	1491	1584-3074

PolyA	1846	127	3114-3240
3' ITR	1830	130	3284-3413

[0254] In some embodiments, the AAV particle comprises a nucleotide sequence encoding a wildtype GBA1 protein, wherein the nucleotide sequence comprises the sequence of SEQ ID NO: 2002 or a sequence that is at least 93% identical thereto.

[0255] In some embodiments, the AAV particle comprising the nucleotide sequence of SEQ ID NO: 2002 further comprises an ITR sequence that is 130 nucleotides in length, wherein, optionally, the ITR sequence comprises the nucleotide sequence of SEQ ID NO: 1829 or SEQ ID NO: 1830 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto. In some embodiments, the AAV particle comprises a 5' ITR comprising the nucleotide sequence of SEQ ID NO: 1829 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto and/or a 3' ITR comprising the nucleotide sequence of SEQ ID NO: 1830 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

[0256] In some embodiments, the AAV particle comprising the nucleotide sequence of SEQ ID NO: 2002 further comprises a CMVie sequence and/or CB promoter operably linked to the nucleotide sequence encoding a GBA1 protein, wherein, optionally, the CMVie sequence comprising the nucleotide sequence of SEQ ID NO: 1831 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto, and the CB promoter comprises the nucleotide sequence of SEQ ID NO: 1834 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

[0257] In some embodiments, the AAV particle comprising the nucleotide sequence of SEQ ID NO: 2002 further comprises a sequence encoding a signal peptide, wherein the sequence encoding the signal peptide comprises the nucleotide sequence of 2005 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical

thereto, wherein the sequence encoding the signal peptide is 5' to the sequence encoding the GBA1 protein.

[0258] In some embodiments, the AAV particle comprising the nucleotide sequence of SEQ ID NO: 2002 further comprises an intron region comprising the nucleotide sequence of SEQ ID NO: 1842 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

[0259] In some embodiments, the AAV particle comprising the nucleotide sequence of SEQ ID NO: 2002 further comprises a polyA sequence comprising the nucleotide sequence of SEQ ID NO: 1846 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

[0260] In some embodiments, the AAV particle comprising the nucleotide sequence of SEQ ID NO: 2002 further comprises, from 5' to 3', one or more of, e.g., all of, an ITR comprising the nucleotide sequence of SEQ ID NO: 1829 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; a CMV_{ie} sequence comprising the nucleotide sequence of SEQ ID NO: 1831 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; a CB promoter comprising the nucleotide sequence of SEQ ID NO: 1834 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; an intron comprising the nucleotide sequence of SEQ ID NO: 1842 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; a signal sequence comprising the nucleotide sequence of SEQ ID NO: 2005 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; a polyA sequence comprising the nucleotide sequence of SEQ ID NO: 1846; and/or an ITR comprising the nucleotide sequence of SEQ ID NO: 1830 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

[0261] In some embodiments, the AAV particle comprises a viral genome comprising a sequence encoding a GBA1 protein, wherein the sequence comprises SEQ ID NO: 2002 or a sequence that is at least 93% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto). In some embodiments, the AAV particle comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 2006 (GBA_VG35) or a nucleotide sequence that is at least 97% (e.g., at least 97%, at least 98%, or at least 99%) identical thereto. In some embodiments, the viral genome comprising the nucleotide sequence of SEQ ID NO: 2006 comprises in 5' to 3' order: a 5' ITR sequence region comprising the nucleotide sequence of SEQ ID NO: 1829, or a nucleotide sequence at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; a CMVie enhancer comprising the nucleotide sequence of SEQ ID NO: 1831, or a nucleotide sequence at least 95% identical thereto; a CB promoter comprising the nucleotide sequence of SEQ ID NO: 1834, or a nucleotide sequence at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; an intron comprising the nucleotide sequence of SEQ ID NO: 1842, or a nucleotide sequence at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; a nucleotide sequence encoding a signal sequence comprising the nucleotide sequence of SEQ ID NO: 2005, or a nucleotide sequence at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; a nucleotide sequence encoding a GBA1 protein comprising the nucleotide sequence of SEQ ID NO: 2002 or a nucleotide sequence at least 94% (e.g., at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the nucleotide sequence of SEQ ID NO: 2002; a polyadenylation sequence comprising the nucleotide sequence of SEQ ID NO: 1846, or a nucleotide sequence at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; and a 3' ITR sequence region comprising the nucleotide sequence of SEQ ID NO: 1830, or a nucleotide sequence at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

[0262] In some embodiments, the AAV viral genome does not comprise a miR-183 binding site.

[0263] In some embodiments, the nucleotide sequence encoding a GBA1 protein comprises a sequence that is at least 94% identical to SEQ ID NO: 2002. In some embodiments, the nucleotide sequence encoding a GBA protein comprises a sequence that is at least 95% identical to SEQ ID NO: 2002. In some embodiments, the nucleotide sequence encoding a GBA1 protein comprises a sequence that is at least 96% identical to SEQ ID NO: 2002. In some embodiments, the nucleotide sequence encoding a GBA1 protein comprises a sequence that is at least 97%

identical to SEQ ID NO: 2002. In some embodiments, the nucleotide sequence encoding a GBA1 protein comprises a sequence that is at least 98% identical to SEQ ID NO: 2002. In some embodiments, the nucleotide sequence encoding a GBA1 protein comprises a sequence that is at least 99% identical to SEQ ID NO: 2002. In some embodiments, the nucleotide sequence encoding a GBA1 protein comprises SEQ ID NO: 2002.

[0264] In some embodiments, the viral genome comprising the nucleotide sequence of SEQ ID NO: 2006, or a nucleotide sequence that is at least 97% (e.g., at least 97%, at least 98%, or at least 99%) identical thereto, encodes a GBA1 protein comprising the amino acid sequence of SEQ ID NO: 1775.

Table 10. Sequence Regions in ITR-to-ITR Sequences

Sequence Regions	GBA_VG36 (SEQ ID NO: 2007)		
	Region SEQ ID NO	Region length	Position in SEQ ID NO: 2007
5' ITR	1829	130	1-130
CMVie	1831	380	204-583
CB promoter	1834	260	590-849
Intron	1842	566	877-1442
Signal sequence	2005	117	1467-1583
GBA1 Variant 4 coding sequence	2002	1491	1584-3074
miR183 binding site	1847	22	3108-3129
Spacer	1848	8	3130-3137
miR183 binding site	1847	22	3138-3159
Spacer	1848	8	3160-3167
miR183 binding site	1847	22	3168-3189
Spacer	1848	8	3190-3197
miR183 binding site	1847	22	3198-3219
PolyA	1846	127	3272-3398
3' ITR	1830	130	3442-3751

[0265] In some embodiments, the AAV particle comprises a nucleotide sequence encoding a wildtype GBA1 protein, wherein the nucleotide sequence comprises the sequence of SEQ ID NO: 2002 or a sequence that is at least 93% (e.g., at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

[0266] In some embodiments, the AAV particle comprising the sequence of SEQ ID NO: 2002 further comprises at least one miR183 binding site. In some embodiments, the AAV particle comprising the sequence of SEQ ID NO: 2002 further comprises four miR183 binding sites, wherein each miR 183 binding site comprises the sequence of SEQ ID NO: 1847 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least

92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

[0267] In some embodiments, the AAV particle comprising the sequence of SEQ ID NO: 2002 further comprises at least one spacer sequence between two miR binding sites, wherein each spacer sequence comprises the sequence of SEQ ID NO: 1848 or a sequence that is at least 75% identical thereto (e.g., at least 75%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto).

[0268] In some embodiments, the AAV particle comprising the sequence of SEQ ID NO: 2002 further comprises a miR binding site series comprising SEQ ID NO: 1849 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

[0269] In some embodiments, the AAV particle comprising the nucleotide sequence of SEQ ID NO: 2002 further comprises, from 5' to 3', one or more of, e.g., all of, an ITR comprising the nucleotide sequence of SEQ ID NO: 1829 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; a CMVie sequence comprising the nucleotide sequence of SEQ ID NO: 1831 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; a CB promoter comprising the nucleotide sequence of SEQ ID NO: 1834 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; an intron comprising the nucleotide sequence of SEQ ID NO: 1842 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; a signal sequence comprising the nucleotide sequence of SEQ ID NO: 2005 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; a miR183 binding site series comprising the nucleotide sequence of SEQ ID NO: 1849 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto; a polyA sequence comprising the

nucleotide sequence of SEQ ID NO: 1846; and/or an ITR comprising the nucleotide sequence of SEQ ID NO: 1830 or a sequence that is at least 70% (e.g., at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical thereto.

[0270] In some embodiments, the AAV particle comprises a viral genome comprising a sequence encoding a GBA1 protein, wherein the sequence comprises SEQ ID NO: 2002 or a sequence that is at least 93% identical thereto (e.g., at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto). In some embodiments, the AAV particle comprises a viral genome comprising the nucleotide sequence of SEQ ID NO: 2007 (GBA_VG36) or a nucleotide sequence that is at least 97% identical thereto (e.g., at least 97%, at least 98%, or at least 99% identical thereto). In some embodiments, the viral genome comprising the nucleotide sequence of SEQ ID NO: 2007 comprises in 5' to 3' order: a 5' ITR sequence region comprising the nucleotide sequence of SEQ ID NO: 1829, or a nucleotide sequence at least 95% identical thereto (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto); a CMVie enhancer comprising the nucleotide sequence of SEQ ID NO: 1831, or a nucleotide sequence at least 95% identical thereto (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto); a CB promoter comprising the nucleotide sequence of SEQ ID NO: 1834, or a nucleotide sequence at least 95% identical thereto (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto); an intron comprising the nucleotide sequence of SEQ ID NO: 1842, or a nucleotide sequence at least 95% identical thereto (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto); a nucleotide sequence encoding a signal sequence comprising the nucleotide sequence of SEQ ID NO: 2005, or a nucleotide sequence at least 95% identical thereto (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto); a nucleotide sequence encoding a GBA1 protein comprising the nucleotide sequence of SEQ ID NO: 2002 or a nucleotide sequence at least 93% (e.g., at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the nucleotide sequence of SEQ ID NO: 2002; a miR183 binding site series comprising the sequence of SEQ ID NO: 1849 or a sequence that is at least 95% identical thereto (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto); a polyadenylation sequence comprising the nucleotide sequence of SEQ ID NO: 1846, or a nucleotide sequence at least 95% identical thereto (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto); and a 3' ITR sequence region comprising the nucleotide sequence of SEQ ID NO: 1830, or a nucleotide sequence at least 95% identical

thereto (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto).

[0271] In some embodiments, the viral genome comprising the nucleotide sequence of SEQ ID NO: 2007, or a nucleotide sequence that is at least 97% identical thereto (e.g., at least 97%, at least 98%, or at least 99% identical thereto), encodes a GBA1 protein comprising the amino acid sequence of SEQ ID NO: 1775.

[0272] In some embodiments, the AAV viral genome further comprises a nucleic acid encoding a capsid protein, e.g., a structural protein. In some embodiments, the capsid protein comprises a VP1 polypeptide, a VP2 polypeptide, and/or a VP3 polypeptide. In some embodiments, the VP1 polypeptide, the VP2 polypeptide, and/or the VP3 polypeptide are encoded by at least one Cap gene. In some embodiments, the AAV viral genome further comprises a nucleic acid encoding a Rep protein, e.g., a non-structural protein. In some embodiments, the Rep protein comprises a Rep78 protein, a Rep68, Rep52 protein, and/or a Rep40 protein. In some embodiments, the Rep78 protein, the Rep68 protein, the Rep52 protein, and/or the Rep40 protein are encoded by at least one Rep gene.

[0273] In some embodiment, the AAV particle comprising a viral genome comprising the nucleotide sequences of SEQ ID NO: 2006 or SEQ ID NO: 2007, or a sequence having at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 2006 or SEQ ID NO: 2007. In some embodiments, the viral genome is packaged in a capsid protein having a serotype or a functional variant thereof selected from Table 1. In some embodiments, the capsid protein comprise a VOY101, VOY201, AAVPHP.N (PHP.N), AAVPHP.B (PHP.B), AAVPHP.A (PHP.A), PHP.B2, PHP.B3, G2B4, G2B5, AAV5, AAV9, AAVrh10, or a functional variant thereof. In some embodiments, the capsid protein comprises a VOY101 capsid protein, or functional variant thereof. In some embodiments, the capsid protein comprises an AAV9 capsid protein, or functional variant thereof. In some embodiments, the capsid protein comprises an AAV5 capsid protein, or functional variant thereof.

[0274] In some embodiments, the AAV particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 2006 or SEQ ID NO: 2007, or a sequence having at least 97%, at least 98%, or at least 99% sequence identity thereto comprises a capsid protein comprising the amino acid sequence of SEQ ID NO: 138, or a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identical thereto. In some embodiments, the capsid protein comprises an amino acid sequence having at least one, two, or three modifications, but not more than 30, 20 or 10 modifications of the amino acid sequence of SEQ ID NO: 138. In some

embodiments, the capsid protein is encoded by the nucleotide sequence of SEQ ID NO: 137, or a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto. In some embodiments, the capsid protein comprises an amino acid substitution at position K449, e.g., a K449R substitution, numbered according to SEQ ID NO:138. In some embodiments, the capsid protein comprises an insert comprising the amino acid sequence of TLAVPFK (SEQ ID NO: 1262), wherein the insert is present immediately subsequent to position 588, relative to a reference sequence numbered according to SEQ ID NO:138. In some embodiments, the capsid protein comprises an amino acid other than "A" at position 587 and/or an amino acid other than "Q" at position 588, numbered according to SEQ ID NO:138. In some embodiments, the capsid protein comprises the amino acid substitution of A587D and/or Q588G, numbered according to SEQ ID NO: 138. In some embodiments, the capsid protein comprises an insert comprising the amino acid sequence PLNGAVHLY (SEQ ID NO: 3648), wherein the amino acid sequence of PLNGAVHLY (SEQ ID NO: 3648) is present immediately subsequent to position 586, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid comprises the amino acid sequence of SEQ ID NO: 3636.

[0275] In some embodiments, the AAV particle comprising a viral genome comprising SEQ ID NO: 2006 or SEQ ID NO: 2007, or a sequence having at least 97%, at least 98%, or at least 99% identity thereto, comprises a capsid protein comprising the amino acid sequence of SEQ ID NO: 1, or a sequence substantially identical (e.g., having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) thereto. In some embodiments, the capsid protein comprises an amino acid sequence having at least one, two or three modifications, but not more than 30, not more than 20 or not more than 10 modifications of the amino acid sequence of SEQ ID NO: 1. In some embodiments, the capsid protein is encoded by the nucleotide sequence of SEQ ID NO: 2, or a nucleotide sequence substantially identical (e.g., having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) thereto.

[0276] The present disclosure provides in some embodiments, vectors, cells, and/or AAV particles comprising the above identified viral genomes.

Self-Complementary and Single Strand Vectors

[0277] In some embodiments, the AAV vector used in the present disclosure is a single

strand vector (ssAAV).

[0278] In some embodiments, the AAV vectors may be self-complementary AAV vectors (scAAVs). *See, e.g.*, US Patent No. 7,465,583. scAAV vectors contain both DNA strands that anneal together to form double stranded DNA. By skipping second strand synthesis, scAAVs allow for rapid expression in the cell.

[0279] In some embodiments, the AAV vector used in the present disclosure is a scAAV.

[0280] Methods for producing and/or modifying AAV vectors are disclosed in the art such as pseudotyped AAV vectors (International Patent Publication Nos. WO200028004; WO200123001; WO2004112727; WO 2005005610 and WO 2005072364, the content of each of which are incorporated herein by reference in their entirety).

Viral Genome Size

[0281] In some embodiments, the viral genome of the AAV particles of the present disclosure may be single or double stranded. The size of the vector genome may be small, medium, large or the maximum size.

[0282] In some embodiments, the vector genome, which comprises a nucleic acid sequence encoding GCaP protein described herein, may be a small single stranded vector genome. A small single stranded vector genome may be about 2.7 kb to about 3.5 kb in size such as about 2.7, about 2.8, about 2.9, about 3.0, about 3.1, about 3.2, about 3.3, about 3.4, or about 3.5 kb in size. In some embodiments, the small single stranded vector genome may be 3.2 kb in size.

[0283] In some embodiments, the vector genome, which comprises a nucleic acid sequence encoding GCaP protein described herein, may be a small double stranded vector genome. A small double stranded vector genome may be about 1.3 to about 1.7 kb in size such as about 1.3, about 1.4, about 1.5, about 1.6, or about 1.7 kb in size. In some embodiments, the small double stranded vector genome may be 1.6 kb in size.

[0284] In some embodiments, the vector genome, which comprises a nucleic acid sequence encoding GCaP protein described herein, may be a medium single stranded vector genome. A medium single stranded vector genome may be about 3.6 to about 4.3 kb in size such as about 3.6, about 3.7, about 3.8, about 3.9, about 4.0, about 4.1, about 4.2, or about 4.3 kb in size. In some embodiments, the medium single stranded vector genome may be 4.0 kb in size.

[0285] In some embodiments, the vector genome, which comprises a nucleic acid sequence encoding GCaP protein described herein, may be a medium double stranded vector genome. A medium double stranded vector genome may be about 1.8 to about 2.1 kb in size such as about 1.8, about 1.9, about 2.0, or about 2.1 kb in size. In some embodiments, the medium double stranded vector genome may be 2.0 kb in size. Additionally, the vector genome may comprise a

promoter and a polyA tail.

[0286] In some embodiments, the vector genome which comprises a nucleic acid sequence encoding GCaSe protein described herein may be a large single stranded vector genome. A large single stranded vector genome may be 4.4 to 6.0 kb in size such as about 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9 and 6.0 kb in size. As a non-limiting example, the large single stranded vector genome may be 4.7 kb in size. As another non-limiting example, the large single stranded vector genome may be 4.8 kb in size. As yet another non-limiting example, the large single stranded vector genome may be 6.0 kb in size.

[0287] In some embodiments, the vector genome which comprises a nucleic acid sequence encoding GCaSe protein described herein may be a large double stranded vector genome. A large double stranded vector genome may be 2.2 to 3.0 kb in size such as about 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 and 3.0 kb in size. As a non-limiting example, the large double stranded vector genome may be 2.4 kb in size.

Backbone

[0288] In certain embodiments, a cis-element such as a vector backbone is incorporated into the viral particle encoding, *e.g.*, a GBA1 protein or a GBA1 protein and an enhancement element described herein. Without wishing to be bound by theory, it is believed, in some embodiments, the backbone sequence may contribute to the stability of GBA1 protein expression, and/or the level of expression of the GBA1 protein.

[0289] The present disclosure also provides in some embodiments, a nucleic acid encoding a viral genome, *e.g.*, a viral genome comprising the nucleotide sequence of any of the viral genomes in Tables 18-21 or 29-32, or a nucleotide sequence substantially identical (*e.g.*, having at least 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100%, sequence identity) thereto, an a backbone region suitable for replication of the viral genome in a cell, *e.g.*, a bacterial cell (*e.g.*, wherein the backbone region comprises one or both of a bacterial origin of replication and a selectable marker).

II. Viral production

General Viral Production Process

[0290] Cells for the production of AAV, *e.g.*, rAAV, particles may comprise, in some embodiments, mammalian cells (such as HEK293 cells) and/or insect cells (such as Sf9 cells).

[0291] In various embodiments, AAV production includes processes and methods for producing AAV particles and vectors which can contact a target cell to deliver a payload, *e.g.* a recombinant viral construct, which includes a nucleotide encoding a payload molecule. In certain embodiments, the viral vectors are adeno-associated viral (AAV) vectors such as

recombinant adeno-associated viral (rAAV) vectors. In certain embodiments, the AAV particles are adeno-associated viral (AAV) particles such as recombinant adeno-associated viral (rAAV) particles.

[0292] In some embodiments, disclosed herein is a vector comprising a viral genome of the present disclosure. In some embodiments, disclosed herein is a cell comprising a viral genome of the present disclosure. In some embodiments, the cell is a bacterial cell, a mammalian cell (e.g., a HEK293 cell), or an insect cell (e.g., an Sf9 cell).

[0293] In some embodiments, disclosed herein is a method of making a viral genome. The method comprising providing a nucleic acid encoding a viral genome described herein and a backbone region suitable for replication of the viral genome in a cell, e.g., a bacterial cell (e.g., wherein the backbone region comprises one or both of a bacterial origin of replication and a selectable marker), and excising the viral genome from the backbone region, e.g., by cleaving the nucleic acid molecule at upstream and downstream of the viral genome. In some embodiments, the viral genome comprising a promoter operably linked to nucleic acid comprising a transgene encoding a GBA1 protein (e.g., a GBA1 protein described herein), will be incorporated into an AAV particle produced in the cell. In some embodiments, the cell is a bacterial cell, a mammalian cell (e.g., a HEK293 cell), or an insect cell (e.g., an Sf9 cell).

[0294] In some embodiments, disclosed herein is a method of making a recombinant AAV particle of the present disclosure, the method comprising (i) providing a host cell comprising a viral genome described herein and incubating the host cell under conditions suitable to enclose the viral genome in a capsid protein, e.g., a capsid protein described herein (e.g., a capsid protein listed in Table 1, e.g., a VOY101 capsid protein or functional variant thereof), thereby making the recombinant AAV particle. In some embodiments, the method comprises prior to step (i), introducing a first nucleic acid comprising the viral genome into a cell. In some embodiments, the host cell comprises a second nucleic acid encoding the capsid protein. In some embodiments, the second nucleic acid is introduced into the host cell prior to, concurrently with, or after the first nucleic acid molecule. In some embodiments, the host cell is a bacterial cell, a mammalian cell (e.g., a HEK293 cell), or an insect cell (e.g., an Sf9 cell).

[0295] In various embodiments, methods are provided herein of producing AAV particles or vectors by (a) contacting a viral production cell with one or more viral expression constructs encoding at least one AAV capsid protein, and one or more payload constructs encoding a payload molecule, which can be selected from: a transgene, a polynucleotide encoding protein, and a modulatory nucleic acid; (b) culturing the viral production cell under conditions such that at least one AAV particle or vector is produced, and (c) isolating the AAV particle or vector

from the production stream.

[0296] In these methods, a viral expression construct may encode at least one structural protein and/or at least one non-structural protein. The structural protein may include any of the native or wild type capsid proteins VP1, VP2, and/or VP3, or a chimeric protein thereof. The non-structural protein may include any of the native or wild type Rep78, Rep68, Rep52, and/or Rep40 proteins or a chimeric protein thereof.

[0297] In certain embodiments, contacting occurs via transient transfection, viral transduction, and/or electroporation.

[0298] In certain embodiments, the viral production cell is selected from a mammalian cell and an insect cell. In certain embodiments, the insect cell includes a *Spodoptera frugiperda* insect cell. In certain embodiments, the insect cell includes a Sf9 insect cell. In certain embodiments, the insect cell includes a Sf21 insect cell.

[0299] The payload construct vector of the present disclosure may include, in various embodiments, at least one inverted terminal repeat (ITR) and may include mammalian DNA.

[0300] Also provided are AAV particles and viral vectors produced according to the methods described herein.

[0301] In various embodiments, the AAV particles of the present disclosure may be formulated as a pharmaceutical composition with one or more acceptable excipients.

[0302] In certain embodiments, an AAV particle or viral vector may be produced by a method described herein.

[0303] In certain embodiments, the AAV particles may be produced by contacting a viral production cell (*e.g.*, an insect cell or a mammalian cell) with at least one viral expression construct encoding at least one capsid protein and at least one payload construct vector. The viral production cell may be contacted by transient transfection, viral transduction, and/or electroporation. The payload construct vector may include a payload construct encoding a payload molecule such as, but not limited to, a transgene, a polynucleotide encoding protein, and a modulatory nucleic acid. The viral production cell can be cultured under conditions such that at least one AAV particle or vector is produced, isolated (*e.g.*, using temperature-induced lysis, mechanical lysis and/or chemical lysis) and/or purified (*e.g.*, using filtration, chromatography, and/or immunoaffinity purification). As a non-limiting example, the payload construct vector may include mammalian DNA.

[0304] In certain embodiments, the AAV particles are produced in an insect cell (*e.g.*, *Spodoptera frugiperda* (Sf9) cell) using a method described herein. As a non-limiting example, the insect cell is contacted using viral transduction which may include baculoviral transduction.

[0305] In certain embodiments, the AAV particles are produced in a mammalian cell (*e.g.*, HEK293 cell) using a method described herein. As a non-limiting example, the mammalian cell is contacted using viral transduction which may include multiplasmid transient transfection (such as triple plasmid transient transfection).

[0306] In certain embodiments, the AAV particle production method described herein produces greater than 10^1 , greater than 10^2 , greater than 10^3 , greater than 10^4 , or greater than 10^5 AAV particles in a viral production cell.

[0307] In certain embodiments, a process of the present disclosure includes production of viral particles in a viral production cell using a viral production system which includes at least one viral expression construct and at least one payload construct. The at least one viral expression construct and at least one payload construct can be co-transfected (*e.g.* dual transfection, triple transfection) into a viral production cell. The transfection is completed using standard molecular biology techniques known and routinely performed by a person skilled in the art. The viral production cell provides the cellular machinery necessary for expression of the proteins and other biomaterials necessary for producing the AAV particles, including Rep proteins which replicate the payload construct and Cap proteins which assemble to form a capsid that encloses the replicated payload constructs. The resulting AAV particle is extracted from the viral production cells and processed into a pharmaceutical preparation for administration.

[0308] In various embodiments, once administered, an AAV particle disclosed herein may, without being bound by theory, contact a target cell and enter the cell, *e.g.*, in an endosome. The AAV particles, *e.g.*, those released from the endosome, may subsequently contact the nucleus of the target cell to deliver the payload construct. The payload construct, *e.g.* recombinant viral construct, may be delivered to the nucleus of the target cell wherein the payload molecule encoded by the payload construct may be expressed.

[0309] In certain embodiments, the process for production of viral particles utilizes seed cultures of viral production cells that include one or more baculoviruses (*e.g.*, a Baculoviral Expression Vector (BEV) or a baculovirus infected insect cell (BIIC) that has been transfected with a viral expression construct and a payload construct vector). In certain embodiments, the seed cultures are harvested, divided into aliquots and frozen, and may be used at a later time point to initiate an infection of a naïve population of production cells.

[0310] In some embodiments, large scale production of AAV particles utilizes a bioreactor. Without being bound by theory, the use of a bioreactor may allow for the precise measurement and/or control of variables that support the growth and activity of viral production cells such as mass, temperature, mixing conditions (impellor RPM or wave oscillation), CO₂ concentration,

O₂ concentration, gas sparge rates and volumes, gas overlay rates and volumes, pH, Viable Cell Density (VCD), cell viability, cell diameter, and/or optical density (OD). In certain embodiments, the bioreactor is used for batch production in which the entire culture is harvested at an experimentally determined time point and AAV particles are purified. In some embodiments, the bioreactor is used for continuous production in which a portion of the culture is harvested at an experimentally determined time point for purification of AAV particles, and the remaining culture in the bioreactor is refreshed with additional growth media components.

[0311] In various embodiments, AAV viral particles can be extracted from viral production cells in a process which includes cell lysis, clarification, sterilization and purification. Cell lysis includes any process that disrupts the structure of the viral production cell, thereby releasing AAV particles. In certain embodiments, cell lysis may include thermal shock, chemical, or mechanical lysis methods. Clarification can include the gross purification of the mixture of lysed cells, media components, and AAV particles. In certain embodiments, clarification includes centrifugation and/or filtration, including but not limited to depth end, tangential flow, and/or hollow fiber filtration.

[0312] In various embodiments, the end result of viral production is a purified collection of AAV particles which include two components: (1) a payload construct (*e.g.* a recombinant AAV vector genome construct) and (2) a viral capsid.

[0313] In certain embodiments, a viral production system or process of the present disclosure includes steps for producing baculovirus infected insect cells (BIICs) using Viral Production Cells (VPC) and plasmid constructs. Viral Production Cells (VPCs) from a Cell Bank (CB) are thawed and expanded to provide a target working volume and VPC concentration. The resulting pool of VPCs is split into a Rep/Cap VPC pool and a Payload VPC pool. One or more Rep/Cap plasmid constructs (viral expression constructs) are processed into Rep/Cap Bacmid polynucleotides and transfected into the Rep/Cap VPC pool. One or more Payload plasmid constructs (payload constructs) are processed into Payload Bacmid polynucleotides and transfected into the Payload VPC pool. The two VPC pools are incubated to produce P1 Rep/Cap Baculoviral Expression Vectors (BEVs) and P1 Payload BEVs. The two BEV pools are expanded into a collection of Plaques, with a single Plaque being selected for Clonal Plaque (CP) Purification (also referred to as Single Plaque Expansion). The process can include a single CP Purification step or can include multiple CP Purification steps either in series or separated by other processing steps. The one-or-more CP Purification steps provide a CP Rep/Cap BEV pool and a CP Payload BEV pool. These two BEV pools can then be stored and used for future production steps, or they can be then transfected into VPCs to produce a Rep/Cap BIIC pool and

a Payload BIIC pool.

[0314] In certain embodiments, a viral production system or process of the present disclosure includes steps for producing AAV particles using Viral Production Cells (VPC) and baculovirus infected insect cells (BIICs). Viral Production Cells (VPCs) from a Cell Bank (CB) are thawed and expanded to provide a target working volume and VPC concentration. The working volume of Viral Production Cells is seeded into a Production Bioreactor and can be further expanded to a working volume of 200-2000 L with a target VPC concentration for BIIC infection. The working volume of VPCs in the Production Bioreactor is then co-infected with Rep/Cap BIICs and Payload BIICs, with a target VPC:BIIC ratio and a target BIIC:BIIC ratio. VCD infection can also utilize BEVs. The co-infected VPCs are incubated and expanded in the Production Bioreactor to produce a bulk harvest of AAV particles and VPCs.

Viral Expression Constructs

[0315] In various embodiments, the viral production system of the present disclosure includes one or more viral expression constructs that can be transfected/transduced into a viral production cell. In certain embodiments, a viral expression construct or a payload construct of the present disclosure can be a bacmid, also known as a baculovirus plasmid or recombinant baculovirus genome. In certain embodiments, the viral expression includes a protein-coding nucleotide sequence and at least one expression control sequence for expression in a viral production cell. In certain embodiments, the viral expression includes a protein-coding nucleotide sequence operably linked to least one expression control sequence for expression in a viral production cell. In certain embodiments, the viral expression construct contains parvoviral genes under control of one or more promoters. Parvoviral genes can include nucleotide sequences encoding non-structural AAV replication proteins, such as Rep genes which encode Rep52, Rep40, Rep68, or Rep78 proteins. Parvoviral genes can include nucleotide sequences encoding structural AAV proteins, such as Cap genes which encode VP1, VP2, and VP3 proteins.

[0316] Viral expression constructs of the present disclosure may include any compound or formulation, biological or chemical, which facilitates transformation, transfection, or transduction of a cell with a nucleic acid. Exemplary biological viral expression constructs include plasmids, linear nucleic acid molecules, and recombinant viruses including baculovirus. Exemplary chemical vectors include lipid complexes. Viral expression constructs are used to incorporate nucleic acid sequences into virus replication cells in accordance with the present disclosure. (O'Reilly, David R., Lois K. Miller, and Verne A. Luckow. *Baculovirus expression vectors: a laboratory manual*. Oxford University Press, 1994.); Maniatis *et al.*, eds. *Molecular*

Cloning. CSH Laboratory, NY, N.Y. (1982); and, Philpott and Schluter, eds. Liposomes as tools in Basic Research and Industry. CRC Press, Ann Arbor, Mich. (1995), the contents of each of which are herein incorporated by reference in their entirety as related to viral expression constructs and uses thereof.

[0317] In certain embodiments, the viral expression construct is an AAV expression construct which includes one or more nucleotide sequences encoding non-structural AAV replication proteins, structural AAV capsid proteins, or a combination thereof.

[0318] In certain embodiments, the viral expression construct of the present disclosure may be a plasmid vector. In certain embodiments, the viral expression construct of the present disclosure may be a baculoviral construct.

[0319] The present disclosure is not limited by the number of viral expression constructs employed to produce AAV particles or viral vectors. In certain embodiments, one, two, three, four, five, six, or more viral expression constructs can be employed to produce AAV particles in viral production cells in accordance with the present disclosure. In certain embodiments of the present disclosure, a viral expression construct may be used for the production of an AAV particles in insect cells. In certain embodiments, modifications may be made to the wild type AAV sequences of the capsid and/or rep genes, for example to improve attributes of the viral particle, such as increased infectivity or specificity, or to enhance production yields.

[0320] In certain embodiments, the viral expression construct may contain a nucleotide sequence which includes start codon region, such as a sequence encoding AAV capsid proteins which include one or more start codon regions. In certain embodiments, the start codon region can be within an expression control sequence. The start codon can be ATG or a non-ATG codon (*i.e.*, a suboptimal start codon where the start codon of the AAV VP1 capsid protein is a non-ATG).

[0321] In certain embodiments, the viral expression construct used for AAV production may contain a nucleotide sequence encoding the AAV capsid proteins where the initiation codon of the AAV VP1 capsid protein is a non-ATG, *i.e.*, a suboptimal initiation codon, allowing the expression of a modified ratio of the viral capsid proteins in the production system, to provide improved infectivity of the host cell. In a non-limiting example, a viral construct vector may contain a nucleic acid construct comprising a nucleotide sequence encoding AAV VP1, VP2, and VP3 capsid proteins, wherein the initiation codon for translation of the AAV VP1 capsid protein is CTG, TTG, or GTG, as described in US Patent No. US 8,163,543, the contents of which are herein incorporated by reference in their entirety as related to AAV capsid proteins and the production thereof.

[0322] In certain embodiments, the viral expression construct of the present disclosure may be a plasmid vector or a baculoviral construct that encodes the parvoviral rep proteins for expression in insect cells. In certain embodiments, a single coding sequence is used for the Rep78 and Rep52 proteins, wherein start codon for translation of the Rep78 protein is a suboptimal start codon, selected from the group consisting of ACG, TTG, CTG, and GTG, that effects partial exon skipping upon expression in insect cells, as described in US Patent No. 8,512,981, the contents of which are herein incorporated by reference in their entirety, for example to promote less abundant expression of Rep78 as compared to Rep52, which may promote high vector yields.

[0323] In certain embodiments, a VP-coding region encodes one or more AAV capsid proteins of a specific AAV serotype. The AAV serotypes for VP-coding regions can be the same or different. In certain embodiments, a VP-coding region can be codon optimized. In certain embodiments, a VP-coding region or nucleotide sequence can be codon optimized for a mammal cell. In certain embodiments, a VP-coding region or nucleotide sequence can be codon optimized for an insect cell. In certain embodiments, a VP-coding region or nucleotide sequence can be codon optimized for a *Spodoptera frugiperda* cell. In certain embodiments, a VP-coding region or nucleotide sequence can be codon optimized for Sf9 or Sf21 cell lines.

[0324] In certain embodiments, a nucleotide sequence encoding one or more VP capsid proteins can be codon optimized to have a nucleotide homology with the reference nucleotide sequence of less than 100%. In certain embodiments, the nucleotide homology between the codon-optimized VP nucleotide sequence and the reference VP nucleotide sequence is less than 100%, less than 99%, less than 98%, less than 97%, less than 96%, less than 95%, less than 94%, less than 93%, less than 92%, less than 91%, less than 90%, less than 89%, less than 88%, less than 87%, less than 86%, less than 85%, less than 84%, less than 83%, less than 82%, less than 81%, less than 80%, less than 78%, less than 76%, less than 74%, less than 72%, less than 70%, less than 68%, less than 66%, less than 64%, less than 62%, less than 60%, less than 55%, less than 50%, and less than 40%.

[0325] In certain embodiments, a viral expression construct or a payload construct of the present disclosure can be a bacmid, also known as a baculovirus plasmid or recombinant baculovirus genome. In certain embodiments, a viral expression construct or a payload construct of the present disclosure (*e.g.* bacmid) can include a polynucleotide incorporated by homologous recombination (transposon donor/acceptor system) into the bacmid by standard molecular biology techniques known and performed by a person skilled in the art.

[0326] In certain embodiments, the polynucleotide incorporated into the bacmid (*i.e.*

polynucleotide insert) can include an expression control sequence operably linked to a protein-coding nucleotide sequence. In certain embodiments, the polynucleotide incorporated into the bacmid can include an expression control sequence which includes a promoter, such as p10 or polh, and which is operably linked to a nucleotide sequence which encodes a structural AAV capsid protein (*e.g.* VP1, VP2, VP3 or a combination thereof). In certain embodiments, the polynucleotide incorporated into the bacmid can include an expression control sequence which includes a promoter, such as p10 or polh, and which is operably linked to a nucleotide sequence which encodes a non-structural AAV capsid protein (*e.g.* Rep78, Rep52, or a combination thereof).

[0327] The method of the present disclosure is not limited by the use of specific expression control sequences. However, when a certain stoichiometry of VP products are achieved (close to 1:1:10 for VP1, VP2, and VP3, respectively) and also when the levels of Rep52 or Rep40 (also referred to as the p19 Reps) are significantly higher than Rep78 or Rep68 (also referred to as the p5 Reps), improved yields of AAV in production cells (such as insect cells) may be obtained. In certain embodiments, the p5/p19 ratio is below 0.6 more, below 0.4, or below 0.3, but always at least 0.03. These ratios can be measured at the level of the protein or can be implicated from the relative levels of specific mRNAs.

[0328] In certain embodiments, AAV particles are produced in viral production cells (such as mammalian or insect cells) wherein all three VP proteins are expressed at a stoichiometry approaching, about or which is: 1:1:10 (VP1:VP2:VP3); 2:2:10 (VP1:VP2:VP3); 2:0:10 (VP1:VP2:VP3); 1-2:0-2:10 (VP1:VP2:VP3); 1-2:1-2:10 (VP1:VP2:VP3); 2-3:0-3:10 (VP1:VP2:VP3); 2-3:2-3:10 (VP1:VP2:VP3); 3:3:10 (VP1:VP2:VP3); 3-5:0-5:10 (VP1:VP2:VP3); or 3-5:3-5:10 (VP1:VP2:VP3).

[0329] In certain embodiments, the expression control regions are engineered to produce a VP1:VP2:VP3 ratio selected from the group consisting of: about or exactly 1:0:10; about or exactly 1:1:10; about or exactly 2:1:10; about or exactly 2:1:10; about or exactly 2:2:10; about or exactly 3:0:10; about or exactly 3:1:10; about or exactly 3:2:10; about or exactly 3:3:10; about or exactly 4:0:10; about or exactly 4:1:10; about or exactly 4:2:10; about or exactly 4:3:10; about or exactly 4:4:10; about or exactly 5:5:10; about or exactly 1-2:0-2:10; about or exactly 1-2:1-2:10; about or exactly 1-3:0-3:10; about or exactly 1-3:1-3:10; about or exactly 1-4:0-4:10; about or exactly 1-4:1-4:10; about or exactly 1-5:1-5:10; about or exactly 2-3:0-3:10; about or exactly 2-3:2-3:10; about or exactly 2-4:2-4:10; about or exactly 2-5:2-5:10; about or exactly 3-4:3-4:10; about or exactly 3-5:3-5:10; and about or exactly 4-5:4-5:10.

[0330] In certain embodiments of the present disclosure, Rep52 or Rep78 is transcribed from

the baculoviral derived polyhedron promoter (polh). Rep52 or Rep78 can also be transcribed from a weaker promoter, for example a deletion mutant of the ie-1 promoter, the Δ ie-1 promoter, has about 20% of the transcriptional activity of that ie-1 promoter. A promoter substantially homologous to the Δ ie-1 promoter may be used. In respect to promoters, a homology of at least 50%, 60%, 70%, 80%, 90% or more, is considered to be a substantially homologous promoter.

Mammalian Cells

[0331] Viral production of the present disclosure disclosed herein describes processes and methods for producing AAV particles or viral vector that contacts a target cell to deliver a payload construct, *e.g.* a recombinant AAV particle or viral construct, which includes a nucleotide encoding a payload molecule. The viral production cell may be selected from any biological organism, including prokaryotic (*e.g.*, bacterial) cells, and eukaryotic cells, including, insect cells, yeast cells and mammalian cells.

[0332] In certain embodiments, the AAV particles of the present disclosure may be produced in a viral production cell that includes a mammalian cell. Viral production cells may comprise mammalian cells such as A549, WEH1, 3T3, 10T1/2, BHK, MDCK, COS 1, COS 7, BSC 1, BSC 40, BMT 10, VERO, W138, HeLa, HEK293, HEK293T (293T), Saos, C2C12, L cells, HT1080, Huh7, HepG2, C127, 3T3, CHO, HeLa cells, KB cells, BHK and primary fibroblast, hepatocyte, and myoblast cells derived from mammals. Viral production cells can include cells derived from any mammalian species including, but not limited to, human, monkey, mouse, rat, rabbit, and hamster or cell type, including but not limited to fibroblast, hepatocyte, tumor cell, cell line transformed cell, *etc.*

[0333] AAV viral production cells commonly used for production of recombinant AAV particles include, but is not limited to other mammalian cell lines as described in U.S. Pat. Nos. 6,156,303, 5,387,484, 5,741,683, 5,691,176, 6,428,988 and 5,688,676; U.S. patent application 2002/0081721, and International Patent Publication Nos. WO 00/47757, WO 00/24916, and WO 96/17947, the contents of each of which are herein incorporated by reference in their entireties insofar as they do not conflict with the present disclosure. In certain embodiments, the AAV viral production cells are trans-complementing packaging cell lines that provide functions deleted from a replication-defective helper virus, *e.g.*, HEK293 cells or other Ea trans-complementing cells.

[0334] In certain embodiments, the packaging cell line 293-10-3 (ATCC Accession No. PTA-2361) may be used to produce the AAV particles, as described in US Patent No. US 6,281,010, the contents of which are herein incorporated by reference in their entirety as related to the 293-10-3 packaging cell line and uses thereof.

[0335] In certain embodiments, of the present disclosure a cell line, such as a HeLa cell line, for trans-complementing E1 deleted adenoviral vectors, which encoding adenovirus E1a and adenovirus E1b under the control of a phosphoglycerate kinase (PGK) promoter can be used for AAV particle production as described in US Patent No. 6365394, the contents of which are incorporated herein by reference in their entirety as related to the HeLa cell line and uses thereof.

[0336] In certain embodiments, AAV particles are produced in mammalian cells using a multiplasmid transient transfection method (such as triple plasmid transient transfection). In certain embodiments, the multiplasmid transient transfection method includes transfection of the following three different constructs: (i) a payload construct, (ii) a Rep/Cap construct (parvoviral Rep and parvoviral Cap), and (iii) a helper construct. In certain embodiments, the triple transfection method of the three components of AAV particle production may be utilized to produce small lots of virus for assays including transduction efficiency, target tissue (tropism) evaluation, and stability. In certain embodiments, the triple transfection method of the three components of AAV particle production may be utilized to produce large lots of materials for clinical or commercial applications.

[0337] AAV particles to be formulated may be produced by triple transfection or baculovirus mediated virus production, or any other method known in the art. Any suitable permissive or packaging cell known in the art may be employed to produce the vectors. In certain embodiments, trans-complementing packaging cell lines are used that provide functions deleted from a replication-defective helper virus, *e.g.*, 293 cells or other E1a trans-complementing cells.

[0338] The gene cassette may contain some or all of the parvovirus (*e.g.*, AAV) cap and rep genes. In certain embodiments, some or all of the cap and rep functions are provided in trans by introducing a packaging vector(s) encoding the capsid and/or Rep proteins into the cell. In certain embodiments, the gene cassette does not encode the capsid or Rep proteins. Alternatively, a packaging cell line is used that is stably transformed to express the cap and/or rep genes.

[0339] Recombinant AAV virus particles are, in certain embodiments, produced and purified from culture supernatants according to the procedure as described in US2016/0032254, the contents of which are incorporated by reference in their entirety as related to the production and processing of recombinant AAV virus particles. Production may also involve methods known in the art including those using 293T cells, triple transfection or any suitable production method.

[0340] In certain embodiments, mammalian viral production cells (*e.g.* 293T cells) can be in an adhesion/adherent state (*e.g.* with calcium phosphate) or a suspension state (*e.g.* with

polyethylenimine (PEI)). The mammalian viral production cell is transfected with plasmids required for production of AAV, (*i.e.*, AAV rep/cap construct, an adenoviral helper construct, and/or ITR flanked payload construct). In certain embodiments, the transfection process can include optional medium changes (*e.g.* medium changes for cells in adhesion form, no medium changes for cells in suspension form, medium changes for cells in suspension form if desired). In certain embodiments, the transfection process can include transfection mediums such as DMEM or F17. In certain embodiments, the transfection medium can include serum or can be serum-free (*e.g.* cells in adhesion state with calcium phosphate and with serum, cells in suspension state with PEI and without serum).

[0341] Cells can subsequently be collected by scraping (adherent form) and/or pelleting (suspension form and scraped adherent form) and transferred into a receptacle. Collection steps can be repeated as necessary for full collection of produced cells. Next, cell lysis can be achieved by consecutive freeze-thaw cycles (-80C to 37C), chemical lysis (such as adding detergent triton), mechanical lysis, or by allowing the cell culture to degrade after reaching ~0% viability. Cellular debris is removed by centrifugation and/or depth filtration. The samples are quantified for AAV particles by DNase resistant genome titration by DNA qPCR.

[0342] AAV particle titers are measured according to genome copy number (genome particles per milliliter). Genome particle concentrations are based on DNA qPCR of the vector DNA as previously reported (Clark *et al.* (1999) Hum. Gene Ther., 10:1031-1039; Veldwijk *et al.* (2002) Mol. Ther., 6:272-278, the contents of which are each incorporated by reference in their entireties as related to the measurement of particle concentrations).

Insect cells

[0343] Viral production of the present disclosure includes processes and methods for producing AAV particles or viral vectors that contact a target cell to deliver a payload construct, *e.g.*, a recombinant viral construct, which includes a nucleotide encoding a payload molecule. In certain embodiments, the AAV particles or viral vectors of the present disclosure may be produced in a viral production cell that includes an insect cell.

[0344] Growing conditions for insect cells in culture, and production of heterologous products in insect cells in culture are well-known in the art, see U.S. Pat. No. 6,204,059, the contents of which are herein incorporated by reference in their entirety as related to the growth and use of insect cells in viral production.

[0345] Any insect cell which allows for replication of parvovirus and which can be maintained in culture can be used in accordance with the present disclosure. AAV viral production cells commonly used for production of recombinant AAV particles include, but is

not limited to, *Spodoptera frugiperda*, including, but not limited to the Sf9 or Sf21 cell lines, *Drosophila* cell lines, or mosquito cell lines, such as *Aedes albopictus* derived cell lines. Use of insect cells for expression of heterologous proteins is well documented, as are methods of introducing nucleic acids, such as vectors, *e.g.*, insect-cell compatible vectors, into such cells and methods of maintaining such cells in culture. *See*, for example, *Methods in Molecular Biology*, ed. Richard, Humana Press, NJ (1995); O'Reilly *et al.*, *Baculovirus Expression Vectors, A Laboratory Manual*, Oxford Univ. Press (1994); Samulski *et al.*, *J. Vir.* 63:3822-8 (1989); Kajigaya *et al.*, *Proc. Nat'l. Acad. Sci. USA* 88: 4646-50 (1991); Ruffing *et al.*, *J. Vir.* 66:6922-30 (1992); Kimbauer *et al.*, *Vir.* 219:37-44 (1996); Zhao *et al.*, *Vir.* 272:382-93 (2000); and Samulski *et al.*, U.S. Pat. No. 6,204,059, the contents of each of which are herein incorporated by reference in their entirety as related to the use of insect cells in viral production.

[0346] In some embodiments, the AAV particles are made using the methods described in WO2015/191508, the contents of which are herein incorporated by reference in their entirety insofar as they do not conflict with the present disclosure.

[0347] In certain embodiments, insect host cell systems, in combination with baculoviral systems (*e.g.*, as described by Luckow *et al.*, *Bio/Technology* 6: 47 (1988)) may be used. In certain embodiments, an expression system for preparing chimeric peptide is *Trichoplusia ni*, Tn 5B1-4 insect cells/baculoviral system, which can be used for high levels of proteins, as described in US Patent No. 6660521, the contents of which are herein incorporated by reference in their entirety as related to the production of viral particles.

[0348] Expansion, culturing, transfection, infection and storage of insect cells can be carried out in any cell culture media, cell transfection media or storage media known in the art, including Hyclone™ SFX-Insect™ Cell Culture Media, Expression System ESF AF™ Insect Cell Culture Medium, ThermoFisher Sf-900II™ media, ThermoFisher Sf-900III™ media, or ThermoFisher Grace's Insect Media. Insect cell mixtures of the present disclosure can also include any of the formulation additives or elements described in the present disclosure, including (but not limited to) salts, acids, bases, buffers, surfactants (such as Poloxamer 188/Pluronic F-68), and other known culture media elements. Formulation additives can be incorporated gradually or as "spikes" (incorporation of large volumes in a short time).

Baculovirus-production systems

[0349] In certain embodiments, processes of the present disclosure can include production of AAV particles or viral vectors in a baculoviral system using a viral expression construct and a payload construct vector. In certain embodiments, the baculoviral system includes Baculovirus expression vectors (BEVs) and/or baculovirus infected insect cells (BIICs). In certain

embodiments, a viral expression construct or a payload construct of the present disclosure can be a bacmid, also known as a baculovirus plasmid or recombinant baculovirus genome. In certain embodiments, a viral expression construct or a payload construct of the present disclosure can be polynucleotide incorporated by homologous recombination (transposon donor/acceptor system) into a bacmid by standard molecular biology techniques known and performed by a person skilled in the art. Transfection of separate viral replication cell populations produces two or more groups (*e.g.* two, three) of baculoviruses (BEVs), one or more group which can include the viral expression construct (Expression BEV), and one or more group which can include the payload construct (Payload BEV). The baculoviruses may be used to infect a viral production cell for production of AAV particles or viral vector.

[0350] In certain embodiments, the process includes transfection of a single viral replication cell population to produce a single baculovirus (BEV) group which includes both the viral expression construct and the payload construct. These baculoviruses may be used to infect a viral production cell for production of AAV particles or viral vector.

[0351] In certain embodiments, BEVs are produced using a Bacmid Transfection agent, such as Promega FuGENE® HD, WFI water, or ThermoFisher Cellfectin® II Reagent. In certain embodiments, BEVs are produced and expanded in viral production cells, such as an insect cell.

[0352] In certain embodiments, the method utilizes seed cultures of viral production cells that include one or more BEVs, including baculovirus infected insect cells (BIICs). The seed BIICs have been transfected/transduced/infected with an Expression BEV which includes a viral expression construct, and also a Payload BEV which includes a payload construct. In certain embodiments, the seed cultures are harvested, divided into aliquots and frozen, and may be used at a later time to initiate transfection/transduction/infection of a naïve population of production cells. In certain embodiments, a bank of seed BIICs is stored at -80 °C or in LN2 vapor.

[0353] Baculoviruses are made of several essential proteins which are essential for the function and replication of the Baculovirus, such as replication proteins, envelope proteins and capsid proteins. The Baculovirus genome thus includes several essential-gene nucleotide sequences encoding the essential proteins. As a non-limiting example, the genome can include an essential-gene region which includes an essential-gene nucleotide sequence encoding an essential protein for the Baculovirus construct. The essential protein can include: GP64 baculovirus envelope protein, VP39 baculovirus capsid protein, or other similar essential proteins for the Baculovirus construct.

[0354] Baculovirus expression vectors (BEV) for producing AAV particles in insect cells, including but not limited to *Spodoptera frugiperda* (Sf9) cells, provide high titers of viral vector

product. Recombinant baculovirus encoding the viral expression construct and payload construct initiates a productive infection of viral vector replicating cells. Infectious baculovirus particles released from the primary infection secondarily infect additional cells in the culture, exponentially infecting the entire cell culture population in a number of infection cycles that is a function of the initial multiplicity of infection, *see* Urabe, M. *et al.* J Virol. 2006 Feb;80(4):1874-85, the contents of which are herein incorporated by reference in their entirety as related to the production and use of BEVs and viral particles.

[0355] Production of AAV particles with baculovirus in an insect cell system may address known baculovirus genetic and physical instability.

[0356] In certain embodiments, the production system of the present disclosure addresses baculovirus instability over multiple passages by utilizing a titerless infected-cells preservation and scale-up system. Small scale seed cultures of viral producing cells are transfected with viral expression constructs encoding the structural and/or non-structural components of the AAV particles. Baculovirus-infected viral producing cells are harvested into aliquots that may be cryopreserved in liquid nitrogen; the aliquots retain viability and infectivity for infection of large scale viral producing cell culture. Wasilko DJ *et al.* Protein Expr Purif. 2009 Jun;65(2):122-32, the contents of which are herein incorporated by reference in their entirety as related to the production and use of BEVs and viral particles.

[0357] A genetically stable baculovirus may be used to produce a source of the one or more of the components for producing AAV particles in invertebrate cells. In certain embodiments, defective baculovirus expression vectors may be maintained episomally in insect cells. In such embodiments, the corresponding bacmid vector is engineered with replication control elements, including but not limited to promoters, enhancers, and/or cell-cycle regulated replication elements.

[0358] In certain embodiments, stable viral producing cells permissive for baculovirus infection are engineered with at least one stable integrated copy of any of the elements necessary for AAV replication and vector production including, but not limited to, the entire AAV genome, Rep and Cap genes, Rep genes, Cap genes, each Rep protein as a separate transcription cassette, each VP protein as a separate transcription cassette, the AAP (assembly activation protein), or at least one of the baculovirus helper genes with native or non-native promoters.

[0359] In some embodiments, the AAV particle of the present disclosure may be produced in insect cells (*e.g.*, Sf9 cells).

[0360] In some embodiments, the AAV particle of the present disclosure may be produced using triple transfection.

[0361] In some embodiments, the AAV particle of the present disclosure may be produced in mammalian cells.

[0362] In some embodiments, the AAV particle of the present disclosure may be produced by triple transfection in mammalian cells.

[0363] In some embodiments, the AAV particle of the present disclosure may be produced by triple transfection in HEK293 cells.

[0364] The AAV viral genomes encoding GCase protein described herein may be useful in the fields of human disease, veterinary applications and a variety of *in vivo* and *in vitro* settings. The AAV particles of the present disclosure may be useful in the field of medicine for the treatment, prophylaxis, palliation, or amelioration of neurological or neuromuscular diseases and/or disorders. In some embodiments, the AAV particles of the disclosure are used for the prevention and/or treatment of GBA1-related disorders.

[0365] Various embodiments of the disclosure herein provide a pharmaceutical composition comprising the AAV particle described herein and a pharmaceutically acceptable excipient.

[0366] Various embodiments of the disclosure herein provide a method of treating a subject in need thereof comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition described herein.

[0367] Certain embodiments of the method provide that the subject is treated by a route of administration of the pharmaceutical composition selected from the group consisting of: intravenous, intracerebroventricular, intraparenchymal, intrathecal, subpial, and intramuscular, or a combination thereof. Certain embodiments of the method provide that the subject is treated for GBA1-related disorders and/or other neurological disorder arising from a deficiency in the quantity or function of GBA1 gene products. In one aspect of the method, a pathological feature of the GBA1-related disorders or the other neurological disorder is alleviated and/or the progression of the GBA1-related disorders or the other neurological disorder is halted, slowed, ameliorated, or reversed.

[0368] Various embodiments of the disclosure herein describe a method of increasing the level of GCase protein in the central nervous system of a subject in need thereof comprising administering to said subject via infusion, an effective amount of the pharmaceutical composition described herein.

[0369] Also described herein are compositions, methods, processes, kits and devices for the design, preparation, manufacture and/or formulation of AAV particles. In some embodiments, payloads, such as but not limited to payloads comprising GCase protein, may be encoded by payload constructs or contained within plasmids or vectors or recombinant adeno-associated

viruses (AAVs).

[0370] The present disclosure also provides administration and/or delivery methods for vectors and viral particles, *e.g.*, AAV particles, for the treatment or amelioration of GBA1-related disorders. Such methods may involve gene replacement or gene activation. Such outcomes are achieved by utilizing the methods and compositions taught herein.

III. Pharmaceutical Compositions

[0371] The present disclosure additionally provides a method for treating GBA1-related disorders and disorders related to deficiencies in the function or expression of GCase protein(s) in a mammalian subject, including a human subject, comprising administering to the subject any of the AAV polynucleotides or AAV genomes described herein (*e.g.*, “vector genomes,” “viral genomes,” or “VGs”) or administering to the subject a particle comprising said AAV polynucleotide or AAV genome, or administering to the subject any of the described compositions, including pharmaceutical compositions.

[0372] As used herein the term “composition” comprises an AAV polynucleotide or AAV genome or AAV particle and at least one excipient.

[0373] As used herein the term “pharmaceutical composition” comprises an AAV polynucleotide or AAV genome or AAV particle and one or more pharmaceutically acceptable excipients.

[0374] Although the descriptions of pharmaceutical compositions, *e.g.*, AAV comprising a payload encoding a GCase protein to be delivered, provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other animal, *e.g.*, to non-human animals, *e.g.* non-human mammals. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys.

[0375] In some embodiments, compositions are administered to humans, human patients, or subjects.

[0376] In some embodiments, the AAV particle formulations described herein may contain a

nucleic acid encoding at least one payload. In some embodiments, the formulations may contain a nucleic acid encoding 1, 2, 3, 4, or 5 payloads. In some embodiments, the formulation may contain a nucleic acid encoding a payload construct encoding proteins selected from categories such as, but not limited to, human proteins, veterinary proteins, bacterial proteins, biological proteins, antibodies, immunogenic proteins, therapeutic peptides and proteins, secreted proteins, plasma membrane proteins, cytoplasmic proteins, cytoskeletal proteins, intracellular membrane bound proteins, nuclear proteins, proteins associated with human disease, and/or proteins associated with non-human diseases. In some embodiments, the formulation contains at least three payload constructs encoding proteins. Certain embodiments provide that at least one of the payloads is GCase protein or a variant thereof.

[0377] A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a “unit dose” refers to a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

IV. Formulations

[0378] Formulations of the AAV pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0379] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the disclosure will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered.

[0380] For example, the composition may comprise between 0.1% and 99% (w/w) of the active ingredient. By way of example, the composition may comprise between 0.1% and 100%, *e.g.*, between .5% and 50%, between 1-30%, between 5-80%, or at least 80% (w/w) active ingredient.

[0381] The AAV particles of the disclosure can be formulated using one or more excipients to: (1) increase stability; (2) increase cell transfection or transduction; (3) permit the sustained or

delayed release; (4) alter the biodistribution (*e.g.*, target the viral particle to specific tissues or cell types); (5) increase the translation of encoded protein *in vivo*; (6) alter the release profile of encoded protein *in vivo* and/or (7) allow for regulatable expression of the payload.

[0382] Formulations of the present disclosure can include, without limitation, saline, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with viral vectors (*e.g.*, for transplantation into a subject), nanoparticle mimics and combinations thereof. Further, the viral vectors of the present disclosure may be formulated using self-assembled nucleic acid nanoparticles.

[0383] In some embodiments, the viral vectors encoding GCa6 protein may be formulated to optimize baricity and/or osmolality. In some embodiments, the baricity and/or osmolality of the formulation may be optimized to ensure optimal drug distribution in the central nervous system or a region or component of the central nervous system.

[0384] In some embodiments, the AAV particles of the disclosure may be formulated in PBS with 0.001% of pluronic acid (F-68) at a pH of about 7.0.

[0385] In some embodiments, the AAV particles of the disclosure may be formulated in PBS, in combination with an ethylene oxide/propylene oxide copolymer (also known as pluronic or poloxamer).

[0386] In some embodiments, the AAV particles of the disclosure may be formulated in PBS with 0.001% pluronic acid (F-68) (poloxamer 188) at a pH of about 7.0.

[0387] In some embodiments, the AAV particles of the disclosure may be formulated in PBS with 0.001% pluronic acid (F-68) (poloxamer 188) at a pH of about 7.3.

[0388] In some embodiments, the AAV particles of the disclosure may be formulated in PBS with 0.001% pluronic acid (F-68) (poloxamer 188) at a pH of about 7.4.

[0389] In some embodiments, the AAV particles of the disclosure may be formulated in a solution comprising sodium chloride, sodium phosphate and an ethylene oxide/propylene oxide copolymer.

[0390] In some embodiments, the AAV particles of the disclosure may be formulated in a solution comprising sodium chloride, sodium phosphate dibasic, potassium chloride, potassium phosphate monobasic, and poloxamer 188/pluronic acid (F-68).

[0391] In some embodiments, the AAV particles of the disclosure may be formulated in a solution comprising 192 mM sodium chloride, 10 mM sodium phosphate (dibasic), 2.7 mM potassium chloride, 2 mM potassium phosphate (monobasic) and 0.001% pluronic F-68 (v/v), at pH 7.4. This formulation is referred to as Formulation 1 in the present disclosure.

[0392] In some embodiments, the AAV particles of the disclosure may be formulated in a

solution comprising about 192 mM sodium chloride, about 10mM sodium phosphate dibasic and about 0.001% poloxamer 188, at a pH of about 7.3. The concentration of sodium chloride in the final solution may be 150 mM-200 mM. As non-limiting examples, the concentration of sodium chloride in the final solution may be 150 mM, 160 mM, 170 mM, 180 mM, 190 mM or 200 mM. The concentration of sodium phosphate dibasic in the final solution may be 1 mM-50 mM. As non-limiting examples, the concentration of sodium phosphate dibasic in the final solution may be 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 40 mM, or 50 mM. The concentration of poloxamer 188 (pluronic acid (F-68)) may be 0.0001%-1%. As non-limiting examples, the concentration of poloxamer 188 (pluronic acid (F-68)) may be 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, or 1%. The final solution may have a pH of 6.8-7.7. Non-limiting examples for the pH of the final solution include a pH of 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, or 7.7.

[0393] In some embodiments, the AAV particles of the disclosure may be formulated in a solution comprising about 1.05% sodium chloride, about 0.212% sodium phosphate dibasic, heptahydrate, about 0.025% sodium phosphate monobasic, monohydrate, and 0.001% poloxamer 188, at a pH of about 7.4. As a non-limiting example, the concentration of AAV particle in this formulated solution may be about 0.001%. The concentration of sodium chloride in the final solution may be 0.1-2.0%, with non-limiting examples of 0.1%, 0.25%, 0.5%, 0.75%, 0.95%, 0.96%, 0.97%, 0.98%, 0.99%, 1.00%, 1.01%, 1.02%, 1.03%, 1.04%, 1.05%, 1.06%, 1.07%, 1.08%, 1.09%, 1.10%, 1.25%, 1.5%, 1.75%, or 2%. The concentration of sodium phosphate dibasic in the final solution may be 0.100-0.300% with non-limiting examples including 0.100%, 0.125%, 0.150%, 0.175%, 0.200%, 0.210%, 0.211%, 0.212%, 0.213%, 0.214%, 0.215%, 0.225%, 0.250%, 0.275%, 0.300%. The concentration of sodium phosphate monobasic in the final solution may be 0.010-0.050%, with non-limiting examples of 0.010%, 0.015%, 0.020%, 0.021%, 0.022%, 0.023%, 0.024%, 0.025%, 0.026%, 0.027%, 0.028%, 0.029%, 0.030%, 0.035%, 0.040%, 0.045%, or 0.050%. The concentration of poloxamer 188 (pluronic acid (F-68)) may be 0.0001%-1%. As non-limiting examples, the concentration of poloxamer 188 (pluronic acid (F-68)) may be 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, or 1%. The final solution may have a pH of 6.8-7.7. Non-limiting examples for the pH of the final solution include a pH of 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, or 7.7.

Excipients

[0394] The formulations of the disclosure can include one or more excipients, each in an amount that together increases the stability of the AAV particle, increases cell transfection or transduction by the viral particle, increases the expression of viral particle encoded protein,

and/or alters the release profile of AAV particle encoded proteins. In some embodiments, a pharmaceutically acceptable excipient may be at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient is approved for use for humans and for veterinary use. In some embodiments, an excipient may be approved by United States Food and Drug Administration. In some embodiments, an excipient may be of pharmaceutical grade. In some embodiments, an excipient may meet the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[0395] Excipients, which, as used herein, include, but are not limited to, any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, and the like, as suited to the particular dosage form desired. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (see Remington: The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro, Lippincott, Williams & Wilkins, Baltimore, MD, 2006; the contents of which are herein incorporated by reference in their entirety). The use of a conventional excipient medium may be contemplated within the scope of the present disclosure, except insofar as any conventional excipient medium may be incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition.

Inactive Ingredients

[0396] In some embodiments, AAV formulations may comprise at least one excipient which is an inactive ingredient. As used herein, the term “inactive ingredient” refers to one or more agents that do not contribute to the activity of the pharmaceutical composition included in formulations. In some embodiments, all, none, or some of the inactive ingredients which may be used in the formulations of the present disclosure may be approved by the US Food and Drug Administration (FDA).

[0397] Formulations of AAV particles disclosed herein may include cations or anions. In some embodiments, the formulations include metal cations such as, but not limited to, Zn^{2+} , Ca^{2+} , Cu^{2+} , Mg^{+} , or combinations thereof. In some embodiments, formulations may include polymers or polynucleotides complexed with a metal cation (see, e.g., U.S. Pat. Nos. 6,265,389 and 6,555,525, the contents of each of which are herein incorporated by reference in their entirety).

V. Uses and Applications

[0398] The compositions of the disclosure may be administered to a subject or used in the manufacture of a medicament for administration to a subject having a deficiency in the quantity or function of GCase protein or having a disease or condition associated with decreased GCase protein expression. As used herein, “associated with decreased GCase protein levels” or “associated with decreased expression” means that one or more symptoms of a disease are caused by lower-than-normal GCase protein levels in a target tissue or in a biofluid such as blood. A disease or condition associated with decreased GCase protein levels or expression may be a disorder of the central nervous system. Also specifically contemplated herein are Parkinson’s Disease and related disorders arising from expression of defective GBA1 gene product, e.g., a PD associated with a GBA1 mutation. Such a disease or condition may be a neuromuscular or a neurological disorder or condition. For example, a disease associated with decreased GCase protein levels may be Parkinson’s Disease or a related disorder, or may be another neurological or neuromuscular disorder described herein, e.g., a PD associated with one or more GBA1 mutations, Gaucher Disease (GD) (e.g., Type 1 GD, Type 2 GD, or Type 3 GD), dementia with Lewy Bodies (DLB), Gaucher disease (GD), Spinal muscular atrophy (SMA), Multiple System Atrophy (MSA), or Multiple sclerosis (MS).

[0399] The present disclosure addresses the need for new technologies by providing a GBA1 protein-related treatment deliverable by AAV-based compositions and complexes for the treatment of GBA1-related disorders.

[0400] In some embodiments, the disclosure provides an AAV particle or pharmaceutical composition according to any one of the embodiments disclosed herein for treating a GBA1-related disorder, such as PD, GD, or DLB. In some embodiments, the present disclosure provides the pharmaceutical composition or the AAV particle of any one the embodiments disclosed herein for use in a method of treating a disorder as disclosed herein, such as PD, GD, or DLB.

[0401] In some embodiments, the disclosure provides a method for treating Parkinson’s Disease (PD) or a related disease, e.g., PD with one or more mutations in a GBA1 gene. In certain embodiments, the AAV particles encoding a GBA1 protein may be administered to a subject to treat Parkinson’s Disease, e.g., PD associated with one or more mutations in a GBA1 gene.

[0402] In some embodiments, the disclosure provides a method for treating Gaucher Disease (GD) (e.g., GD1, GD2, or GD3). In some embodiments, the GD is GD1. In some embodiments, the GD is GD3.

[0403] In some embodiments, the disclosure provides a method of treating Dementia with Lewy Bodies (DLB).

[0404] In some embodiments, administration of the AAV particles comprising viral genomes that encode a GBA1 protein may protect central nervous system pathways from degeneration. The compositions and methods described herein are also useful for treating Gaucher disease (such as Type 1 or Type 3 GD), and Dementia with Lewy Bodies, and other GBA1-related disorders.

[0405] In some embodiments, the delivery of the AAV particles may halt or slow progression of GBA1-related disorders as measured by cholesterol accumulation in CNS cells (as determined, for example, by filipin staining and quantification). In certain embodiments, the delivery of the AAV particles improves symptoms of GBA1-related disorders, including, for example, cognitive, muscular, physical, and sensory symptoms of GBA1-related disorders.

[0406] In some embodiments, the present disclosure encompasses the delivery of pharmaceutical, prophylactic, diagnostic, or imaging compositions in combination with agents that may improve their bioavailability, reduce and/or modify their metabolism, and/or modify their distribution within the body.

[0407] In certain embodiments, the pharmaceutical compositions described herein are used as research tools, particularly in *in vitro* investigations using human cell lines such as HEK293T and *in vivo* testing in nonhuman primates which will occur prior to human clinical trials.

CNS diseases

[0408] The present disclosure provides a method for treating a disease, disorder and/or condition in a mammalian subject, including a human subject, comprising administering to the subject any of the viral particles *e.g.*, AAV, AAV particle, or AAV viral genome that produces a GBA1 protein described herein or administering to the subject a particle comprising said AAV particle or AAV genome, or administering to the subject any of the described compositions, including pharmaceutical compositions.

[0409] In some embodiments, AAV particles of the present disclosure, through delivery of a functional payload that is a therapeutic product comprising a GBA1 protein or variant thereof that can modulate the level or function of a gene product in the CNS.

[0410] A functional payload may alleviate or reduce symptoms that result from abnormal level and/or function of a gene product (*e.g.*, an absence or defect in a protein) in a subject in need thereof or that otherwise confers a benefit to a CNS disorder in a subject in need thereof.

[0411] As non-limiting examples, companion or combination therapeutic products delivered by AAV particles of the present disclosure may include, but are not limited to, growth and

trophic factors, cytokines, hormones, neurotransmitters, enzymes, anti-apoptotic factors, angiogenic factors, a GBA1 protein, and any protein known to be mutated in pathological disorders such as GBA1-related disorders.

[0412] In some embodiments, AAV particles of the present disclosure may be used to treat diseases that are associated with impairments of the growth and development of the CNS, *e.g.*, neurodevelopmental disorders. In some aspects, such neurodevelopmental disorders may be caused by genetic mutations.

[0413] In some embodiments, the neurological disorders may be functional neurological disorders with motor and/or sensory symptoms which have neurological origin in the CNS. As non-limiting examples, functional neurological disorders may be chronic pain, seizures, speech problems, involuntary movements, or sleep disturbances.

[0414] In some embodiments, the neurological or neuromuscular disease, disorder, and/or condition is GBA1-related disorders. In some embodiments, the delivery of the AAV particles may halt or slow the disease progression of GBA1-related disorders by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more than 95% using a known analysis method and comparator group for GBA1-related disorders. As a non-limiting example, the delivery of the AAV particles may halt or slow progression of GBA1-related disorders as measured by cholesterol accumulation in CNS cells (as determined, for example, by filipin staining and quantification).

[0415] In some embodiments, the delivery of an AAV particle described herein increases the amount of GBA1 protein in a tissue by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or more than 100%. In some embodiments, the delivery of an AAV particle described herein may increase the amount of GBA1 protein in a tissue to be comparable to (*e.g.*, approximately the same as) the amount of GBA1 protein in the corresponding tissue of a healthy subject. In some embodiments, the delivery of an AAV particle described herein may increase the amount of GBA1 protein in a tissue effective to reduce one or more symptoms of a disease associated with decreased GBA1 protein expression or a deficiency in the quantity and/or function of GBA1 protein.

[0416] In some embodiments, the AAV particles and AAV viral genomes described herein, upon administration to subject or introduction to a target cell, increase GBA1 activity about 2-3 fold over baseline GBA1 activity. In the case of subjects or target cells with deficient GBA1 activity, as in the case of subjects having a GBA1-related disorder or cells or tissues harboring at least one mutation in a GBA1 gene, the AAV particles and AAV vector genomes described

herein restore GBA1 activity to normal levels, as defined by GBA1 activity levels in subjects, tissues, and cells not afflicted with a GBA1-related disorder or not harboring a GBA1 gene mutation. In some embodiments, the AAV particles and AAV vector genomes described herein effectively reduce α -synuclein levels in subjects having a GBA1-related disorder or cells or tissues harboring at least one mutation in a GBA1 gene. In some embodiments, the AAV particles and AAV viral genomes described herein effectively prevent α -synuclein mediated pathology.

Therapeutic applications

[0417] The present disclosure additionally provides methods for treating non-infectious diseases and/or disorders in a mammalian subject, including a human subject, comprising administering to the subject any of the AAV particles or pharmaceutical compositions described herein. In some embodiments, non-infectious diseases and/or disorders treated according to the methods described herein include, but are not limited to, Parkinson's Disease (PD) (e.g., PD associated with one or more mutations in a GBA1 gene), Dementia with Lewy Bodies (DLB), Multiple System Atrophy (MSA), Decreased muscle mass, Spinal muscular atrophy (SMA), Alzheimer's disease (AD), Amyotrophic lateral sclerosis (ALS), Huntington's Disease (HD), Multiple sclerosis (MS), Stroke, Migraine, Pain, Neuropathies, Psychiatric disorders including schizophrenia, bipolar disorder, and autism, Cancer, ocular diseases, systemic diseases of the blood, heart and bone, Immune system and Autoimmune diseases and Inflammatory diseases.

[0418] The present disclosure provides a method for administering to a subject in need thereof, including a human subject, a therapeutically effective amount of the AAV particles of the invention to slow, stop or reverse disease progression. As a non-limiting example, disease progression may be measured by tests or diagnostic tool(s) known to those skilled in the art. As another non-limiting example, disease progression may be measured by change in the pathological features of the brain, CSF, or other tissues or fluids of the subject.

Gaucher Disease

[0419] Homozygous or compound heterozygous GBA1 mutations lead to Gaucher disease ("GD"). See Sardi, S. Pablo, Jesse M. Cedarbaum, and Patrik Brundin. Movement Disorders 33.5 (2018): 684-696, the contents of which are incorporated by reference in their entirety. Gaucher disease is one of the most prevalent lysosomal storage disorders, with an estimated standardized birth incidence in the general population of between 0.4 to 5.8 individuals per 100,000. Heterozygous GBA1 mutations can lead to PD. Indeed, GBA1 mutations occur in 7-10% of total PD patients, making GBA1 mutations the most important genetic risk factor of PD. PD-GBA1 patients have reduced levels of lysosomal enzyme beta-glucocerebrosidase (GCase),

which results in increased accumulations of glycosphingolipid glucosylceramide (GluCer), which in turn is correlated with exacerbated α -Synuclein aggregation and concomitant neurological symptoms. Gaucher disease and PD, as well as other lysosomal storage disorders including Lewy body diseases such as Dementia with Lewy Bodies, and related diseases, in some cases, share common etiology in the GBA1 gene. See Sidransky, E. and Lopez, G. *Lancet Neurol.* 2012 November; 11(11): 986–998, the contents of which are incorporated by reference in their entirety.

[0420] Gaucher disease can present as GD1 (Type 1 GD), which is the most common type of Gaucher disease among Ashkenazi Jewish populations. In some embodiments, a Type I GD is a non-neuronopathic GD (e.g., does not affect the CNS, e.g., impacts cells and tissues outside of the CNS, e.g., a peripheral cell or tissue, e.g., a heart tissue, a liver tissue, a spleen tissue, or a combination thereof). The carrier frequency among Ashkenazi Jewish populations is approximately 1 in 12 individuals. GD2 (Type 2 GD) is characterized by acute neuronopathic GD (e.g., affects the CNS, e.g., cells and tissues of the brain, spinal cord, or both), and has an estimated incidence of 1 in 150,000 live births. GD2 (Type 2 GD) is an early onset disease, typically presenting at about 1 year of age. Visceral involvement is extensive and severe, with numerous attributes of CNS disease, including oculomotor dysfunction, and bulbar palsy and generalized weakness, and progressive development delay. GD2 progresses to severe hypertonia, rigidity, opisthotonos, dysphagia, and seizures, typically resulting in death before age 2. GD3 (Type 3 GD) is characterized by sub-acute neuropathic GD and as an estimated incidence of 1 in 200,000 live births. GD3 typically presents with pronounced neurologic signs, including a characteristic mask-like face, strabismus, supranuclear gaze palsy, and poor upward gaze initiation. GD2 and GD3 are each further characterized as associated with progressive encephalopathy, with developmental delay, cognitive impairment, progressive dementia, ataxia, myoclonus, and various gaze palsies. GD1, on the other hand, can have variable etiology, with visceromegaly, marrow and skeletal and pulmonary pathology, bleeding diatheses, and developmental delay. GD is further associated with increased rates of hematologic malignancies.

[0421] Deficiency of Glucocerebrosidase (GCase) is the underlying mechanism of GD. Low GCase activity leads to accumulation of glucocerebroside and other glycolipids within the lysosomes of macrophages. Accumulation can amount to about 20-fold to about 100-fold higher than in control cells or subjects without GCase deficiency. Pathologic lipid accumulation in macrophages accounts for < 2% of additional tissue mass observed in the liver and spleen of GD patients. Additional increase in organ weight and volume is attributed to an inflammatory and hyperplastic cellular response.

[0422] Current treatments of GD include administration of recombinant enzymes, imiglucerase, taliglucerase alfa, and velaglucerase alfa. However, these intravenous enzyme therapies do not cross the blood brain barrier (BBB), and are not suitable for treatment of GD with Parkinson's disease or other neuronopathic forms of GD.

Parkinson's Disease

[0423] Parkinson's Disease (PD) is a progressive disorder of the nervous system affecting especially the substantia nigra of the brain. PD develops as a result of the loss of dopamine producing brain cells. Typical early symptoms of PD include shaking or trembling of a limb, *e.g.* hands, arms, legs, feet and face. Additional characteristic symptoms are stiffness of the limbs and torso, slow movement or an inability to move, impaired balance and coordination, cognitive changes, and psychiatric conditions *e.g.* depression and visual hallucinations. PD has both familial and idiopathic forms and it is suggested to be involved with genetic and environmental causes. PD affects more than 4 million people worldwide. In the US, approximately 60,000 cases are identified annually. Generally PD begins at the age of 50 or older. An early-onset form of the condition begins at age younger than 50, and juvenile-onset PD begins before age of 20.

[0424] Death of dopamine producing brain cells related to PD has been associated with aggregation, deposition and dysfunction of alpha-synuclein protein (see, *e.g.* Marques and Outeiro, 2012, *Cell Death Dis.* 3:e350, Jenner, 1989, *J Neurol Neurosurg Psychiatry*. Special Supplement, 22-28, and references therein). Studies have suggested that alpha-synuclein has a role in presynaptic signaling, membrane trafficking and regulation of dopamine release and transport. Alpha-synuclein aggregates, *e.g.* in forms of oligomers, have been suggested to be species responsible for neuronal dysfunction and death. Mutations of the alpha-synuclein gene (SNCA) have been identified in the familial forms of PD, but also environmental factors, *e.g.* neurotoxin affect alpha-synuclein aggregation. Other suggested causes of brain cell death in PD are dysfunction of proteasomal and lysosomal systems, reduced mitochondrial activity.

[0425] PD is related to other diseases related to alpha-synuclein aggregation, referred to as "synucleinopathies." Such diseases include, but are not limited to, Parkinson's Disease Dementia (PDD), multiple system atrophy (MSA), dementia with Lewy bodies, juvenile-onset generalized neuroaxonal dystrophy (Hallervorden-Spatz disease), pure autonomic failure (PAF), neurodegeneration with brain iron accumulation type-1 (NBIA-1) and combined Alzheimer's and Parkinson's disease.

[0426] As of today, no cure or preventative therapy for PD has been identified. A variety of drug therapies available provide relief to the symptoms. Non-limiting examples of symptomatic

medical treatments include carbidopa and levodopa combination reducing stiffness and slow movement, and anticholinergics to reduce trembling and stiffness. Other optional therapies include *e.g.* deep brain stimulation and surgery. There remains a need for therapy affecting the underlying pathophysiology. For example, antibodies targeting alpha-synuclein protein, or other proteins relevant for brain cell death in PD, may be used to prevent and/or treat PD.

[0427] In some embodiment, methods of the present invention may be used to treat subjects suffering from PD (*e.g.*, PD associated with one or more mutations in a GBA1 gene) and other synucleinopathies. In some cases, methods of the present invention may be used to treat subjects suspected of developing PD (*e.g.*, a PD associated with one or more mutations in a GBA1 gene) and other synucleinopathies.

[0428] AAV Particles and methods of using the AAV particles described herein may be used to prevent, manage and/or treat PD, *e.g.*, a PD associated with one or more mutations in a GBA1 gene.

[0429] Approximately 5% of PD patients carry one or more GBA1 mutations: 10% of patients with type 1 GD develop PD before the age of 80 years, compared to about 3–4% in the normal population. Additionally, heterozygous or homozygous GBA1 mutation(s) have been shown to increase the risk of PD 20–30 fold.

Dementia with Lewy Bodies

[0430] Dementia with Lewy Bodies (DLB), also known as diffuse Lewy body disease, is a form of progressive dementia, characterized by cognitive decline, fluctuating alertness and attention, visual hallucinations and parkinsonian motor symptoms. DLB may be inherited by an autosomal dominant pattern. DLB affects more than 1 million individuals in the US. The condition typically shows symptoms at the age of 50 or older.

[0431] DLB is caused by the abnormal build-up of Lewy bodies, aggregates of the alpha-synuclein protein, in the cytoplasm of neurons in the brain areas controlling memory and motor control. The pathophysiology of these aggregates is very similar to aggregates observed in Parkinson's disease and DLB also has similarities to Alzheimer's disease. Inherited DLB has been associated with gene mutation(s) in GBAs.

[0432] As of today, there is no cure or prevention therapy for DLB. A variety of drug therapies available are aimed at managing the cognitive, psychiatric and motor control symptoms of the condition. Non-limiting examples of symptomatic medical treatments include *e.g.* acetylcholinesterase inhibitors to reduce cognitive symptoms, and levodopa to reduce stiffness and loss of movement. There remains a need for therapy affecting the underlying pathophysiology.

[0433] In some embodiments, methods of the present disclosure may be used to treat subjects suffering from DLB (e.g., a DLB associated with one or more mutations in a GBA1 gene). In some cases, the methods may be used to treat subjects suspected of developing DLB (e.g., a DLB associated with one or more mutations in a GBA1 gene).

[0434] AAV Particles and methods of using the AAV particles described in the present invention may be used to prevent, manage and/or treat DLB (e.g., a DLB associated with one or more mutations in a GBA1 gene).

VI. Dosing and Administration

Administration

[0435] In some aspects, the present disclosure provides administration and/or delivery methods for vectors and viral particles, e.g., AAV particles, encoding GCase protein or a variant thereof, for the prevention, treatment, or amelioration of diseases or disorders of the CNS. For example, administration of the AAV particles prevents, treats, or ameliorates GBA1-related disorders. Thus, robust widespread GCase protein distribution throughout the CNS and periphery is desired for maximal efficacy. Particular target tissues for administration or delivery include CNS tissues, brain tissue, and, more specifically, caudate-putamen, thalamus, superior colliculus, cortex, and corpus collosum. Particular embodiments provide administration and/or delivery of the AAV particles and AAV vector genomes described herein to caudate-putamen and/or substantia nigra. Other particular embodiments provide administration and/or delivery of the AAV particles and AAV vector genomes described herein to thalamus.

[0436] The AAV particles of the present disclosure may be administered by any route which results in a therapeutically effective outcome. These include, but are not limited to, enteral (into the intestine), gastroenteral, epidural (into the dura matter), oral (by way of the mouth), transdermal, peridural, intracerebral (into the cerebrum), intracerebroventricular (into the cerebral ventricles), intracranial (into the skull), picutaneous (application onto the skin), intradermal, (into the skin itself), subcutaneous (under the skin), nasal administration (through the nose), intravenous (into a vein), intravenous bolus, intravenous drip, intraarterial (into an artery), intramuscular (into a muscle), intracardiac (into the heart), intraosseous infusion (into the bone marrow), intraparenchymal (into the substance of), intrathecal (into the spinal canal), intraperitoneal, (infusion or injection into the peritoneum), intravesicular infusion, intravitreal, (through the eye), intracavernous injection (into a pathologic cavity) intracavitary (into the base of the penis), intravaginal administration, intrauterine, extra-amniotic administration, transdermal (diffusion through the intact skin for systemic distribution), transmucosal (diffusion through a mucous membrane), transvaginal, insufflation (snorting), sublingual, sublabial, enema,

eye drops (onto the conjunctiva), in ear drops, auricular (in or by way of the ear), buccal (directed toward the cheek), conjunctival, cutaneous, dental (to a tooth or teeth), electro-osmosis, endocervical, endosinusial, endotracheal, extracorporeal, hemodialysis, infiltration, interstitial, intra-abdominal, intra-amniotic, intra-articular, intrabiliary, intrabronchial, intrabursal, intracartilaginous (within a cartilage), intracaudal (within the cauda equine), intracisternal (within the cisterna magna cerebellomedullaris), intracorneal (within the cornea), dental intracoronaral, intracoronary (within the coronary arteries), intracorporus cavernosum (within the dilatable spaces of the corpora cavernosa of the penis), intradiscal (within a disc), intraductal (within a duct of a gland), intraduodenal (within the duodenum), intradural (within or beneath the dura), intraepidermal (to the epidermis), intraesophageal (to the esophagus), intragastric (within the stomach), intragingival (within the gingivae), intraileal (within the distal portion of the small intestine), intralesional (within or introduced directly to a localized lesion), intraluminal (within a lumen of a tube), intralymphatic (within the lymph), intramedullary (within the marrow cavity of a bone), intrameningeal (within the meninges), intraocular (within the eye), intraovarian (within the ovary), intrapericardial (within the pericardium), intrapleural (within the pleura), intraprostatic (within the prostate gland), intrapulmonary (within the lungs or its bronchi), intrasinal (within the nasal or periorbital sinuses), intraspinal (within the vertebral column), intrasynovial (within the synovial cavity of a joint), intratendinous (within a tendon), intratesticular (within the testicle), intrathecal (within the cerebrospinal fluid at any level of the cerebrospinal axis), intrathoracic (within the thorax), intratubular (within the tubules of an organ), intratumor (within a tumor), intratympanic (within the auris media), intravascular (within a vessel or vessels), intraventricular (within a ventricle), iontophoresis (by means of electric current where ions of soluble salts migrate into the tissues of the body), irrigation (to bathe or flush open wounds or body cavities), laryngeal (directly upon the larynx), nasogastric (through the nose and into the stomach), occlusive dressing technique (topical route administration which is then covered by a dressing which occludes the area), ophthalmic (to the external eye), oropharyngeal (directly to the mouth and pharynx), parenteral, percutaneous, periarticular, peridural, perineural, periodontal, rectal, respiratory (within the respiratory tract by inhaling orally or nasally for local or systemic effect), retrobulbar (behind the pons or behind the eyeball), soft tissue, subarachnoid, subconjunctival, submucosal, subpial, topical, transplacental (through or across the placenta), transtracheal (through the wall of the trachea), transtympanic (across or through the tympanic cavity), ureteral (to the ureter), urethral (to the urethra), vaginal, caudal block, diagnostic, nerve block, biliary perfusion, cardiac perfusion, photopheresis or spinal.

[0437] In some embodiments, the AAV particles of the present disclosure are administered intravenously.

[0438] In some embodiments, AAV particles of the present disclosure are administered so as to be delivered to a target cell or tissue. Delivery to a target cell results in GCa6 protein expression. A target cell may be any cell in which it is considered desirable to increase GCa6 protein expression levels. A target cell may be a CNS cell. Non-limiting examples of such cells and/or tissues include, dorsal root ganglia and dorsal columns, proprioceptive sensory neurons, Clark's column, gracile and cuneate nuclei, cerebellar dentate nucleus, corticospinal tracts and the cells comprising the same, Betz cells, and cells of the heart.

[0439] In some embodiments, compositions may be administered in a way that allows them to cross the blood-brain barrier, vascular barrier, or other epithelial barrier.

[0440] In some embodiments, delivery of GCa6 protein by adeno-associated virus (AAV) particles to cells of the central nervous system (*e.g.*, parenchyma) comprises infusion into cerebrospinal fluid (CSF). CSF is produced by specialized ependymal cells that comprise the choroid plexus located in the ventricles of the brain. CSF produced within the brain then circulates and surrounds the central nervous system including the brain and spinal cord. CSF continually circulates around the central nervous system, including the ventricles of the brain and subarachnoid space that surrounds both the brain and spinal cord, while maintaining a homeostatic balance of production and reabsorption into the vascular system. The entire volume of CSF is replaced approximately four to six times per day or approximately once every four hours, though values for individuals may vary.

[0441] In some embodiments, the AAV particles may be delivered by systemic delivery. In some embodiments, the systemic delivery may be by intravascular administration. In some embodiments, the systemic delivery may be by intravenous (IV) administration.

[0442] In some embodiments, the AAV particles may be delivered by intravenous delivery.

[0443] In some embodiments, the AAV particle is administered to the subject via focused ultrasound (FUS), *e.g.*, coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration, *e.g.*, as described in Terstappen *et al.* (Nat Rev Drug Discovery, <https://doi.org/10.1038/s41573-021-00139-y> (2021)), Burgess *et al.* (Expert Rev Neurother. 15(5): 477–491 (2015)), and/or Hsu *et al.* (PLOS One 8(2): 1-8), the contents of which are incorporated herein by reference in its entirety.

[0444] In some embodiments, the AAV particles may be delivered by injection into the CSF pathway. Non-limiting examples of delivery to the CSF pathway include intrathecal and intracerebroventricular administration.

- [0445] In some embodiments, the AAV particles may be delivered by thalamic delivery.
- [0446] In some embodiments, the AAV particles may be delivered by intracerebral delivery.
- [0447] In some embodiments, the AAV particles may be delivered by intracardiac delivery.
- [0448] In some embodiments, the AAV particles may be delivered by intracranial delivery.
- [0449] In some embodiments, the AAV particles may be delivered by intra cisterna magna (ICM) delivery.
- [0450] In some embodiments, the AAV particles may be delivered by direct (intraparenchymal) injection into an organ (*e.g.*, CNS (brain or spinal cord)). In some embodiments, the intraparenchymal delivery may be to any region of the brain or CNS.
- [0451] In some embodiments, the AAV particles may be delivered by intrastriatal injection.
- [0452] In some embodiments, the AAV particles may be delivered into the putamen.
- [0453] In some embodiments, the AAV particles may be delivered into the spinal cord.
- [0454] In some embodiments, the AAV particles of the present disclosure may be administered to the ventricles of the brain.
- [0455] In some embodiments, the AAV particles of the present disclosure may be administered to the ventricles of the brain by intracerebroventricular delivery.
- [0456] In some embodiments, the AAV particles of the present disclosure may be administered by intramuscular delivery.
- [0457] In some embodiments, the AAV particles of the present disclosure are administered by more than one route described above. As a non-limiting example, the AAV particles may be administered by intravenous delivery and thalamic delivery.
- [0458] In some embodiments, the AAV particles of the present disclosure are administered by more than one route described above. As a non-limiting example, the AAV particles may be administered by intravenous delivery and intracerebral delivery.
- [0459] In some embodiments, the AAV particles of the present disclosure are administered by more than one route described above. As a non-limiting example, the AAV particles may be administered by intravenous delivery and intracranial delivery.
- [0460] In some embodiments, the AAV particles of the present disclosure are administered by more than one route described above. In some embodiments, the AAV particles of the present disclosure may be delivered by intrathecal and intracerebroventricular administration.
- [0461] In some embodiments, the AAV particles may be delivered to a subject to improve and/or correct mitochondrial dysfunction.
- [0462] In some embodiments, the AAV particles may be delivered to a subject to preserve neurons. The neurons may be primary and/or secondary sensory neurons. In some embodiments,

AAV particles are delivered to dorsal root ganglia and/or neurons thereof.

[0463] In some embodiments, administration of the AAV particles may preserve and/or correct function in the sensory pathways.

[0464] In some embodiments, the AAV particles may be delivered via intravenous (IV), intracerebroventricular (ICV), intraparenchymal, and/or intrathecal (IT) infusion and the therapeutic agent may also be delivered to a subject via intramuscular (IM) limb infusion in order to deliver the therapeutic agent to the skeletal muscle. Delivery of AAVs by intravascular limb infusion is described by Gruntman and Flotte, Human Gene Therapy Clinical Development, 2015, 26(3), 159-164, the contents of which are herein incorporated by reference in their entirety.

[0465] In some embodiments, delivery of viral vector pharmaceutical compositions in accordance with the present disclosure to cells of the central nervous system (*e.g.*, parenchyma) comprises a rate of delivery defined by $\text{VG/hour} = \text{mL/hour} * \text{VG/mL}$, wherein VG is viral genomes, VG/mL is composition concentration, and mL/hour is rate of infusion.

[0466] In some embodiments, delivery of AAV particle pharmaceutical compositions in accordance with the present disclosure to cells of the central nervous system (*e.g.*, parenchyma) comprises infusion of up to 1 mL. In some embodiments, delivery of viral vector pharmaceutical compositions in accordance with the present disclosure to cells of the central nervous system (*e.g.*, parenchyma) may comprise infusion of 0.0001, 0.0002, 0.001, 0.002, 0.003, 0.004, 0.005, 0.008, 0.010, 0.015, 0.020, 0.025, 0.030, 0.040, 0.050, 0.060, 0.070, 0.080, 0.090, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, or 0.9 mL.

[0467] In some embodiments, delivery of AAV particle pharmaceutical compositions in accordance with the present disclosure to cells of the central nervous system (*e.g.*, parenchyma) comprises infusion of between about 1 mL to about 120 mL. In some embodiments, delivery of viral vector pharmaceutical compositions in accordance with the present disclosure to cells of the central nervous system (*e.g.*, parenchyma) may comprise an infusion of 0.1, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, or 120 mL. In some embodiments delivery of AAV particles to cells of the central nervous system (*e.g.*, parenchyma) comprises infusion of at least 3 mL. In some embodiments, delivery of AAV particles to cells of the central nervous system (*e.g.*, parenchyma) consists of infusion

of 3 mL. In some embodiments, delivery of AAV particles to cells of the central nervous system (*e.g.*, parenchyma) comprises infusion of at least 10 mL. In some embodiments, delivery of AAV particles to cells of the central nervous system (*e.g.*, parenchyma) consists of infusion of 10 mL.

[0468] In some embodiments, the volume of the AAV particle pharmaceutical composition delivered to the cells of the central nervous system (*e.g.*, parenchyma) of a subject is 2 μ l, 20 μ l, 50 μ l, 80 μ l, 100 μ l, 200 μ l, 300 μ l, 400 μ l, 500 μ l, 600 μ l, 700 μ l, 800 μ l, 900 μ l, 1000 μ l, 1100 μ l, 1200 μ l, 1300 μ l, 1400 μ l, 1500 μ l, 1600 μ l, 1700 μ l, 1800 μ l, 1900 μ l, 2000 μ l, or more than 2000 μ l.

[0469] In some embodiments, the volume of the AAV particle pharmaceutical composition delivered to a region in both hemispheres of a subject brain is 2 μ l, 20 μ l, 50 μ l, 80 μ l, 100 μ l, 200 μ l, 300 μ l, 400 μ l, 500 μ l, 600 μ l, 700 μ l, 800 μ l, 900 μ l, 1000 μ l, 1100 μ l, 1200 μ l, 1300 μ l, 1400 μ l, 1500 μ l, 1600 μ l, 1700 μ l, 1800 μ l, 1900 μ l, 2000 μ l, or more than 2000 μ l. In some embodiments, the volume delivered to a region in both hemispheres is 200 μ l. As another non-limiting example, the volume delivered to a region in both hemispheres is 900 μ l. As yet another non-limiting example, the volume delivered to a region in both hemispheres is 1800 μ l.

[0470] In certain embodiments, AAV particle or viral vector pharmaceutical compositions in accordance with the present disclosure may be administered at about 10 to about 600 μ l/site, about 50 to about 500 μ l/site, about 100 to about 400 μ l/site, about 120 to about 300 μ l/site, about 140 to about 200 μ l/site, or about 160 μ l/site.

[0471] In some embodiments, the total volume delivered to a subject may be split between one or more administration sites *e.g.*, 1, 2, 3, 4, 5, or more than 5 sites. In some embodiments, the total volume is split between administration to the left and right hemisphere.

Delivery of AAV Particles

[0472] In some embodiments, the AAV particles or pharmaceutical compositions of the present disclosure may be administered or delivered using the methods for treatment of disease described in US Patent No. 8,999,948, or International Publication No. WO2014178863, the contents of which are herein incorporated by reference in their entirety.

[0473] In some embodiments, the AAV particles or pharmaceutical compositions of the present disclosure may be administered or delivered using the methods for delivering gene therapy in Alzheimer's Disease or other neurodegenerative conditions as described in US Application No. 20150126590, the contents of which are herein incorporated by reference in their entirety.

[0474] In some embodiments, the AAV particles or pharmaceutical compositions of the

present disclosure may be administered or delivered using the methods for delivery of a CNS gene therapy as described in US Patent Nos. 6,436,708, and 8,946,152, and International Publication No. WO2015168666, the contents of which are herein incorporated by reference in their entirety.

[0475] In some embodiments, the AAV particles of the present disclosure may be administered or delivered using the methods for the delivery of AAV virions described in European Patent Application No. EP1857552, the contents of which are herein incorporated by reference in their entirety.

[0476] In some embodiments, the AAV particle or pharmaceutical compositions of the present disclosure may be administered or delivered using the methods for delivering proteins using AAV vectors described in European Patent Application No. EP2678433, the contents of which are herein incorporated by reference in their entirety.

[0477] In some embodiments, the viral vector encoding GCa₆ protein may be administered or delivered using the methods for delivering DNA molecules using AAV vectors described in US Patent No. US 5858351, the contents of which are herein incorporated by reference in their entirety.

[0478] In some embodiments, the AAV particle or pharmaceutical compositions of the present disclosure may be administered or delivered using the methods for delivering DNA to the bloodstream described in US Patent No. US 6,211,163, the contents of which are herein incorporated by reference in their entirety.

[0479] In some embodiments, the viral vector encoding GCa₆ protein may be administered or delivered using the methods for delivering AAV virions described in US Patent No. US 6325998, the contents of which are herein incorporated by reference in their entirety.

[0480] In some embodiments, the viral vector encoding GCa₆ protein may be administered or delivered using the methods for delivering DNA to muscle cells described in US Patent No. US 6335011, the contents of which are herein incorporated by reference in their entirety.

[0481] In some embodiments, the viral vector encoding GCa₆ protein may be administered or delivered using the methods for delivering DNA to muscle cells and tissues described in US Patent No. US 6610290, the contents of which are herein incorporated by reference in their entirety.

[0482] In some embodiments, the viral vector encoding GCa₆ protein may be administered or delivered using the methods for delivering DNA to muscle cells described in US Patent No. US 7704492, the contents of which are herein incorporated by reference in their entirety.

[0483] In some embodiments, the viral vector encoding GCa₆ protein may be administered

or delivered using the methods for delivering a payload to skeletal muscles described in US Patent No. US 7112321, the contents of which are herein incorporated by reference in their entirety.

[0484] In some embodiments, the AAV particle or pharmaceutical compositions of the present disclosure may be administered or delivered using the methods for delivering a payload to the central nervous system described in US Patent No. US 7,588,757, the contents of which are herein incorporated by reference in their entirety.

[0485] In some embodiments, the AAV particle or pharmaceutical compositions of the present disclosure may be administered or delivered using the methods for delivering a payload described in US Patent No. US 8,283,151, the contents of which are herein incorporated by reference in their entirety.

[0486] In some embodiments, the AAV particle or pharmaceutical compositions of the present disclosure may be administered or delivered using the methods for delivering a payload for the treatment of Alzheimer disease described in US Patent No. US 8318687, the contents of which are herein incorporated by reference in their entirety.

[0487] In some embodiments, the AAV particle or pharmaceutical compositions of the present disclosure may be administered or delivered using the methods for delivering a payload described in International Patent Publication No. WO2012144446, the contents of which are herein incorporated by reference in their entirety.

[0488] In some embodiments, the AAV particle or pharmaceutical compositions of the present disclosure may be administered or delivered using the methods for delivering a payload using a glutamic acid decarboxylase (GAD) delivery vector described in International Patent Publication No. WO2001089583, the contents of which are herein incorporated by reference in their entirety.

[0489] In some embodiments, the AAV particle or pharmaceutical compositions of the present disclosure may be administered or delivered using the methods for delivering a payload to neural cells described in International Patent Publication No. WO2012057363, the contents of which are herein incorporated by reference in their entirety.

[0490] In some embodiments, the AAV particle or pharmaceutical compositions of the present disclosure may be administered or delivered using the methods for delivering a payload described in International Patent Publication No. WO2001096587, the contents of which are herein incorporated by reference in their entirety.

[0491] In some embodiments, the AAV particle or pharmaceutical compositions of the present disclosure may be administered or delivered using the methods for delivering a payload

to muscle tissue described in International Patent Publication No. WO2002014487, the contents of which are herein incorporated by reference in their entirety.

[0492] In some embodiments, a catheter may be used to administer the AAV particles. In certain embodiments, the catheter or cannula may be located at more than one site in the spine for multi-site delivery. The viral particles encoding may be delivered in a continuous and/or bolus infusion. Each site of delivery may be a different dosing regimen or the same dosing regimen may be used for each site of delivery. In some embodiments, the sites of delivery may be in the cervical and the lumbar region. In some embodiments, the sites of delivery may be in the cervical region. In some embodiments, the sites of delivery may be in the lumbar region.

[0493] In some embodiments, a subject may be analyzed for spinal anatomy and pathology prior to delivery of the AAV particles described herein. As a non-limiting example, a subject with scoliosis may have a different dosing regimen and/or catheter location compared to a subject without scoliosis.

[0494] In some embodiments, the delivery method and duration is chosen to provide broad transduction in the spinal cord. In some embodiments, intrathecal delivery is used to provide broad transduction along the rostral-caudal length of the spinal cord. In some embodiments, multi-site infusions provide a more uniform transduction along the rostral-caudal length of the spinal cord.

Delivery to Cells

[0495] In some aspects, the present disclosure provides a method of delivering to a cell or tissue any of the above-described AAV particles, comprising contacting the cell or tissue with said AAV particle or contacting the cell or tissue with a formulation comprising said AAV particle, or contacting the cell or tissue with any of the described compositions, including pharmaceutical compositions. The method of delivering the AAV particle to a cell or tissue can be accomplished *in vitro*, *ex vivo*, or *in vivo*.

Delivery to Subjects

[0496] In some aspects, the present disclosure additionally provides a method of delivering to a subject, including a mammalian subject, any of the above-described AAV particles comprising administering to the subject said AAV particle, or administering to the subject a formulation comprising said AAV particle, or administering to the subject any of the described compositions, including pharmaceutical compositions.

[0497] In some embodiments, the AAV particles may be delivered to bypass anatomical blockages such as, but not limited to the blood brain barrier.

[0498] In some embodiments, the AAV particles may be formulated and delivered to a

subject by a route which increases the speed of drug effect as compared to oral delivery.

[0499] In some embodiments, the AAV particles may be delivered by a method to provide uniform transduction of the spinal cord and dorsal root ganglion (DRG). In some embodiments, the AAV particles may be delivered using intrathecal infusion.

[0500] In some embodiments, a subject may be administered the AAV particles described herein using a bolus infusion. As used herein, a “bolus infusion” means a single and rapid infusion of a substance or composition.

[0501] In some embodiments, the AAV particles encoding GCase protein may be delivered in a continuous and/or bolus infusion. Each site of delivery may be a different dosing regimen or the same dosing regimen may be used for each site of delivery. As a non-limiting example, the sites of delivery may be in the cervical and the lumbar region. As another non-limiting example, the sites of delivery may be in the cervical region. As another non-limiting example, the sites of delivery may be in the lumbar region.

[0502] In some embodiments, the AAV particles may be delivered to a subject via a single route administration.

[0503] In some embodiments, the AAV particles may be delivered to a subject via a multi-site route of administration. For example, a subject may be administered the AAV particles at 2, 3, 4, 5, or more than 5 sites.

[0504] In some embodiments, a subject may be administered the AAV particles described herein using sustained delivery over a period of minutes, hours or days. The infusion rate may be changed depending on the subject, distribution, formulation or another delivery parameter known to those in the art.

[0505] In some embodiments, if continuous delivery (continuous infusion) of the AAV particles is used, the continuous infusion may be for 1 hour, 2, hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, or more than 24 hours.

[0506] In some embodiments, the intracranial pressure may be evaluated prior to administration. The route, volume, AAV particle concentration, infusion duration and/or vector titer may be optimized based on the intracranial pressure of a subject.

[0507] In some embodiments, the AAV particles may be delivered by systemic delivery. In some embodiments, the systemic delivery may be by intravascular administration.

[0508] In some embodiments, the AAV particles may be delivered by injection into the CSF pathway. Non-limiting examples of delivery to the CSF pathway include intrathecal and

intracerebroventricular administration.

[0509] In some embodiments, the AAV particles may be delivered by direct (intraparenchymal) injection into the substance of an organ, *e.g.*, one or more regions of the brain.

[0510] In some embodiments, the AAV particles may be delivered by subpial injection into the spinal cord. For example, subjects may be placed into a spinal immobilization apparatus. A dorsal laminectomy may be performed to expose the spinal cord. Guiding tubes and XYZ manipulators may be used to assist catheter placement. Subpial catheters may be placed into the subpial space by advancing the catheter from the guiding tube and AAV particles may be injected through the catheter (Miyanojara *et al.*, Mol Ther Methods Clin Dev. 2016; 3: 16046). In some cases, the AAV particles may be injected into the cervical subpial space. In some cases, the AAV particles may be injected into the thoracic subpial space.

[0511] In some embodiments, the AAV particles may be delivered by direct injection to the CNS of a subject. In some embodiments, direct injection is intracerebral injection, intraparenchymal injection, intrathecal injection, intra-cisterna magna injection, or any combination thereof. In some embodiments, direct injection to the CNS of a subject comprises convection enhanced delivery (CED). In some embodiments, administration comprises peripheral injection. In some embodiments, peripheral injection is intravenous injection.

[0512] In some embodiments, the AAV particles may be delivered to a subject in order to increase the GCase protein levels in the caudate-putamen, thalamus, superior colliculus, cortex, and/or corpus callosum as compared to endogenous levels. The increase may be 0.1x to 5x, 0.5x to 5x, 1x to 5x, 2x to 5x, 3x to 5x, 4x to 5x, 0.1x to 4x, 0.5x to 4x, 1x to 4x, 2x to 4x, 3x to 4x, 0.1x to 3x, 0.5x to 3x, 1x to 3x, 2x to 3x, 0.1x to 2x, 0.5x to 2x, 0.1x to 1x, 0.5x to 1x, 0.1x to 0.5x, 1x to 2x, 0.1x, 0.2x, 0.3x, 0.4x, 0.5x, 0.6x, 0.7x, 0.8x, 0.9x, 1.0x, 1.1x, 1.2x, 1.3x, 1.4x, 1.5x, 1.6x, 1.7x, 1.8x, 1.9x, 2.0x, 2.1x, 2.2x, 2.3x, 2.4x, 2.5x, 2.6x, 2.7x, 2.8x, 2.9x, 3.0x, 3.1x, 3.2x, 3.3x, 3.4x, 3.5x, 3.6x, 3.7x, 3.8x, 3.9x, 4.0x, 4.1x, 4.2x, 4.3x, 4.4x, 4.5x, 4.6x, 4.7x, 4.8x, 4.9x or more than 5x as compared to endogenous levels.

[0513] In some embodiments, the AAV particles may be delivered to a subject in order to increase the GCase protein levels in the caudate, putamen, thalamus, superior colliculus, cortex, and/or corpus callosum by transducing cells in these CNS regions. Transduction may also be referred to as the amount of cells that are positive for GCase protein. The transduction may be greater than or equal to 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of cells in these CNS regions.

[0514] In some embodiments, delivery of AAV particles comprising a viral genome encoding

GCCase protein described herein to neurons in the caudate-putamen, thalamus, superior colliculus, cortex, and/or corpus callosum will lead to an increased expression of GCCase protein. The increased expression may lead to improved survival and function of various cell types in these CNS regions and subsequent improvement of GBA1-related disorder symptoms.

[0515] In particular embodiments, the AAV particles may be delivered to a subject in order to establish widespread distribution of the GCCase throughout the nervous system by administering the AAV particles to the thalamus of the subject.

[0516] Specifically, in some embodiments, the increased expression of GCCase protein may lead to improved gait, sensory capability, coordination of movement and strength, functional capacity, cognition, and/or quality of life.

Dosing

[0517] In some aspects, the present disclosure provides methods comprising administering viral vectors and their payloads in accordance with the disclosure to a subject in need thereof. Viral vector pharmaceutical, imaging, diagnostic, or prophylactic compositions thereof, may be administered to a subject using any amount and any route of administration effective for preventing, treating, diagnosing, or imaging a disease, disorder, and/or condition (*e.g.*, a disease, disorder, and/or condition associated with decreased GCCase protein expression or a deficiency in the quantity and/or function of GCCase protein). In some embodiments, the disease, disorder, and/or condition is GBA1-related disorders. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like. Compositions in accordance with the disclosure are typically formulated in unit dosage form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present disclosure may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex, and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific peptide(s) employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

[0518] In certain embodiments, AAV particle pharmaceutical compositions in accordance with the present disclosure may be administered at dosage levels sufficient to deliver GCCase

protein from about 0.0001 mg/kg to about 100 mg/kg, from about 0.001 mg/kg to about 0.05 mg/kg, from about 0.005 mg/kg to about 0.05 mg/kg, from about 0.001 mg/kg to about 0.005 mg/kg, from about 0.05 mg/kg to about 0.5 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, from about 0.1 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, or from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic, diagnostic, prophylactic, or imaging effect. It will be understood that the above dosing concentrations may be converted to VG or viral genomes per kg or into total viral genomes administered by one of skill in the art.

[0519] In certain embodiments, the desired dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). When multiple administrations are employed, split dosing regimens such as those described herein may be used. As used herein, a “split dose” is the division of single unit dose or total daily dose into two or more doses, *e.g.*, two or more administrations of the single unit dose. As used herein, a “single unit dose” is a dose of any therapeutic composition administered in one dose/at one time/single route/single point of contact, *i.e.*, single administration event. In some embodiments, a single unit dose is provided as a discrete dosage form (*e.g.*, a tablet, capsule, patch, loaded syringe, vial, *etc.*). As used herein, a “total daily dose” is an amount given or prescribed in 24-hour period. It may be administered as a single unit dose. The viral particles may be formulated in buffer only or in a formulation described herein.

[0520] A pharmaceutical composition described herein can be formulated into a dosage form described herein, such as a topical, intranasal, pulmonary, intratracheal, or injectable (*e.g.*, intravenous, intraocular, intravitreal, intramuscular, intracardiac, intraperitoneal, and/or subcutaneous).

[0521] In some embodiments, delivery of the AAV particles described herein results in minimal serious adverse events (SAEs) as a result of the delivery of the AAV particles.

[0522] In some embodiments, delivery of AAV particle pharmaceutical compositions in accordance with the present disclosure to cells of the central nervous system (*e.g.*, parenchyma) may comprise a total concentration between about 1×10^6 VG/mL and about 1×10^{16} VG/mL. In some embodiments, delivery may comprise a composition concentration of about 1×10^6 , 2×10^6 , 3×10^6 , 4×10^6 , 5×10^6 , 6×10^6 , 7×10^6 , 8×10^6 , 9×10^6 , 1×10^7 , 2×10^7 , 3×10^7 , 4×10^7 , 5×10^7 , 6×10^7 , 7×10^7 , 8×10^7 , 9×10^7 , 1×10^8 , 2×10^8 , 3×10^8 , 4×10^8 , 5×10^8 , 6×10^8 , 7×10^8 , 8×10^8 , 9×10^8 , 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 , 9×10^9 , 1×10^{10} , 2×10^{10} , 3×10^{10} , 4×10^{10} , 5×10^{10} ,

6×10^{10} , 7×10^{10} , 8×10^{10} , 9×10^{10} , 1×10^{11} , 1.6×10^{11} , 1.8×10^{11} , 2×10^{11} , 3×10^{11} , 4×10^{11} , 5×10^{11} ,
 5.5×10^{11} , 6×10^{11} , 7×10^{11} , 8×10^{11} , 9×10^{11} , 0.8×10^{12} , 0.83×10^{12} , 1×10^{12} , 1.1×10^{12} , 1.2×10^{12} ,
 1.3×10^{12} , 1.4×10^{12} , 1.5×10^{12} , 1.6×10^{12} , 1.7×10^{12} , 1.8×10^{12} , 1.9×10^{12} , 2×10^{12} , 2.1×10^{12} , 2.2×10^{12} ,
 2.3×10^{12} , 2.4×10^{12} , 2.5×10^{12} , 2.6×10^{12} , 2.7×10^{12} , 2.8×10^{12} , 2.9×10^{12} , 3×10^{12} , 3.1×10^{12} , 3.2×10^{12} ,
 3.3×10^{12} , 3.4×10^{12} , 3.5×10^{12} , 3.6×10^{12} , 3.7×10^{12} , 3.8×10^{12} , 3.9×10^{12} , 4×10^{12} , 4.1×10^{12} , 4.2×10^{12} ,
 4.3×10^{12} , 4.4×10^{12} , 4.5×10^{12} , 4.6×10^{12} , 4.7×10^{12} , 4.8×10^{12} , 4.9×10^{12} , 5×10^{12} , 6×10^{12} , 7×10^{12} ,
 8×10^{12} , 9×10^{12} , 1×10^{13} , 2×10^{13} , 2.3×10^{13} , 3×10^{13} , 4×10^{13} , 5×10^{13} , 6×10^{13} , 7×10^{13} , 8×10^{13} , 9×10^{13} ,
 1×10^{14} , 1.9×10^{14} , 2×10^{14} , 3×10^{14} , 4×10^{14} , 5×10^{14} , 6×10^{14} , 7×10^{14} , 8×10^{14} , 9×10^{14} , 1×10^{15} , 2×10^{15} ,
 3×10^{15} , 4×10^{15} , 5×10^{15} , 6×10^{15} , 7×10^{15} , 8×10^{15} , 9×10^{15} , or 1×10^{16} VG/mL. In some
embodiments, the concentration of the viral vector in the composition is 1×10^{13} VG/mL. In some
embodiments, the concentration of the viral vector in the composition is 1.1×10^{12} VG/mL. In
some embodiments, the concentration of the viral vector in the composition is 3.7×10^{12} VG/mL.
In some embodiments, the concentration of the viral vector in the composition is 8×10^{11} VG/mL.
In some embodiments, the concentration of the viral vector in the composition is 2.6×10^{12}
VG/mL. In some embodiments, the concentration of the viral vector in the composition is
 4.9×10^{12} VG/mL. In some embodiments, the concentration of the viral vector in the composition
is 0.8×10^{12} VG/mL. In some embodiments, the concentration of the viral vector in the
composition is 0.83×10^{12} VG/mL. In some embodiments, the concentration of the viral vector in
the composition is the maximum final dose which can be contained in a vial. In some
embodiments, the concentration of the viral vector in the composition is 1.6×10^{11} VG/mL. In
some embodiments, the concentration of the viral vector in the composition is 5×10^{11} VG/mL. In
some embodiments, the concentration of the viral vector in the composition is 2.3×10^{13} VG/mL.
In some embodiments, the concentration of the viral vector in the composition is 1.9×10^{14}
VG/mL.

[0523] In some embodiments, delivery of AAV particle pharmaceutical compositions in
accordance with the present disclosure to cells of the central nervous system (*e.g.*, parenchyma)
may comprise a total concentration per subject between about 1×10^6 VG and about 1×10^{16} VG.
In some embodiments, delivery may comprise a composition concentration of about 1×10^6 ,
 2×10^6 , 3×10^6 , 4×10^6 , 5×10^6 , 6×10^6 , 7×10^6 , 8×10^6 , 9×10^6 , 1×10^7 , 2×10^7 , 3×10^7 , 4×10^7 , 5×10^7 ,
 6×10^7 , 7×10^7 , 8×10^7 , 9×10^7 , 1×10^8 , 2×10^8 , 3×10^8 , 4×10^8 , 5×10^8 , 6×10^8 , 7×10^8 , 8×10^8 , 9×10^8 ,
 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 , 9×10^9 , 1×10^{10} , 2×10^{10} , 3×10^{10} , 4×10^{10} ,
 5×10^{10} , 6×10^{10} , 7×10^{10} , 8×10^{10} , 9×10^{10} , 1×10^{11} , 1.6×10^{11} , 2×10^{11} , 2.1×10^{11} , 2.2×10^{11} , 2.3×10^{11} ,
 2.4×10^{11} , 2.5×10^{11} , 2.6×10^{11} , 2.7×10^{11} , 2.8×10^{11} , 2.9×10^{11} , 3×10^{11} , 4×10^{11} , 4.6×10^{11} , 5×10^{11} ,
 6×10^{11} , 7×10^{11} , 7.1×10^{11} , 7.2×10^{11} , 7.3×10^{11} , 7.4×10^{11} , 7.5×10^{11} , 7.6×10^{11} , 7.7×10^{11} , 7.8×10^{11} ,

7.9x10¹¹, 8x10¹¹, 9x10¹¹, 1x10¹², 1.1 x10¹², 1.2x10¹², 1.3x10¹², 1.4x10¹², 1.5x10¹², 1.6x10¹², 1.7x10¹², 1.8x10¹², 1.9x10¹², 2x10¹², 2.3x10¹², 3x10¹², 4x10¹², 4.1x10¹², 4.2x10¹², 4.3x10¹², 4.4x10¹², 4.5x10¹², 4.6x10¹², 4.7x10¹², 4.8x10¹², 4.9x10¹², 5x10¹², 6x10¹², 7x10¹², 8x10¹², 8.1x10¹², 8.2x10¹², 8.3x10¹², 8.4x10¹², 8.5x10¹², 8.6x10¹², 8.7x10¹², 8.8 x10¹², 8.9x10¹², 9x10¹², 1x10¹³, 2x10¹³, 3x10¹³, 4x10¹³, 5x10¹³, 6x10¹³, 7x10¹³, 8x10¹³, 9x10¹³, 1x10¹⁴, 2x10¹⁴, 3x10¹⁴, 4x10¹⁴, 5x10¹⁴, 6x10¹⁴, 7x10¹⁴, 8x10¹⁴, 9x10¹⁴, 1x10¹⁵, 2x10¹⁵, 3x10¹⁵, 4x10¹⁵, 5x10¹⁵, 6x10¹⁵, 7x10¹⁵, 8x10¹⁵, 9x10¹⁵, or 1x10¹⁶ VG/subject. In some embodiments, the concentration of the viral vector in the composition is 2.3x10¹¹ VG/ subject. In some embodiments, the concentration of the viral vector in the composition is 7.2x10¹¹ VG/ subject. In some embodiments, the concentration of the viral vector in the composition is 7.5x10¹¹ VG/ subject. In some embodiments, the concentration of the viral vector in the composition is 1.4x10¹² VG/ subject. In some embodiments, the concentration of the viral vector in the composition is 4.8x10¹² VG/ subject. In some embodiments, the concentration of the viral vector in the composition is 8.8x10¹² VG/ subject. In some embodiments, the concentration of the viral vector in the composition is 2.3x10¹² VG/ subject. In some embodiments, the concentration of the viral vector in the composition is 2x10¹⁰ VG/ subject. In some embodiments, the concentration of the viral vector in the composition is 1.6x10¹¹ VG/ subject. In some embodiments, the concentration of the viral vector in the composition is 4.6x10¹¹ VG/ subject.

[0524] In some embodiments, delivery of AAV particles to cells of the central nervous system (*e.g.*, parenchyma) may comprise a total dose between about 1 × 10⁶ VG and about 1 × 10¹⁶ VG. In some embodiments, delivery may comprise a total dose of about 1 × 10⁶, 2 × 10⁶, 3 × 10⁶, 4 × 10⁶, 5 × 10⁶, 6 × 10⁶, 7 × 10⁶, 8 × 10⁶, 9 × 10⁶, 1 × 10⁷, 2 × 10⁷, 3 × 10⁷, 4 × 10⁷, 5 × 10⁷, 6 × 10⁷, 7 × 10⁷, 8 × 10⁷, 9 × 10⁷, 1 × 10⁸, 2 × 10⁸, 3 × 10⁸, 4 × 10⁸, 5 × 10⁸, 6 × 10⁸, 7 × 10⁸, 8 × 10⁸, 9 × 10⁸, 1 × 10⁹, 2 × 10⁹, 3 × 10⁹, 4 × 10⁹, 5 × 10⁹, 6 × 10⁹, 7 × 10⁹, 8 × 10⁹, 9 × 10⁹, 1 × 10¹⁰, 1.9 × 10¹⁰, 2 × 10¹⁰, 3 × 10¹⁰, 3.73 × 10¹⁰, 4 × 10¹⁰, 5 × 10¹⁰, 6 × 10¹⁰, 7 × 10¹⁰, 8 × 10¹⁰, 9 × 10¹⁰, 1 × 10¹¹, 2 × 10¹¹, 2.5 × 10¹¹, 3 × 10¹¹, 4 × 10¹¹, 5 × 10¹¹, 6 × 10¹¹, 7 × 10¹¹, 8 × 10¹¹, 9 × 10¹¹, 1 × 10¹², 2 × 10¹², 3 × 10¹², 4 × 10¹², 5 × 10¹², 6 × 10¹², 7 × 10¹², 8 × 10¹², 9 × 10¹², 1 × 10¹³, 2 × 10¹³, 3 × 10¹³, 4 × 10¹³, 5 × 10¹³, 6 × 10¹³, 7 × 10¹³, 8 × 10¹³, 9 × 10¹³, 1 × 10¹⁴, 2 × 10¹⁴, 3 × 10¹⁴, 4 × 10¹⁴, 5 × 10¹⁴, 6 × 10¹⁴, 7 × 10¹⁴, 8 × 10¹⁴, 9 × 10¹⁴, 1 × 10¹⁵, 2 × 10¹⁵, 3 × 10¹⁵, 4 × 10¹⁵, 5 × 10¹⁵, 6 × 10¹⁵, 7 × 10¹⁵, 8 × 10¹⁵, 9 × 10¹⁵, or 1 × 10¹⁶ VG. In some embodiments, the total dose is 1 × 10¹³ VG. In some embodiments, the total dose is 3 × 10¹³ VG. In some embodiments, the total dose is 3.73 × 10¹⁰ VG. In some embodiments, the total dose is 1.9 × 10¹⁰ VG. In some embodiments, the total dose is 2.5 × 10¹¹ VG. In some embodiments, the total dose is 5 × 10¹¹ VG. In some embodiments, the total dose is 1 × 10¹² VG.

In some embodiments, the total dose is 5×10^{12} VG.

Combinations

[0525] The AAV particles may be used in combination with one or more other therapeutic, prophylactic, diagnostic, or imaging agents. The phrase “in combination with,” is not intended to require that the agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of the present disclosure. Compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. In some embodiments, the present disclosure encompasses the delivery of pharmaceutical, prophylactic, diagnostic, or imaging compositions in combination with agents that may improve their bioavailability, reduce and/or modify their metabolism, and/or modify their distribution within the body.

[0526] The therapeutic agents may be approved by the US Food and Drug Administration or may be in clinical trial or at the preclinical research stage. The therapeutic agents may utilize any therapeutic modality known in the art, with non-limiting examples including gene silencing or interference (*i.e.*, miRNA, siRNA, RNAi, shRNA), gene editing (*i.e.*, TALEN, CRISPR/Cas9 systems, zinc finger nucleases), and gene, protein or enzyme replacement.

Measurement of Expression

[0527] Expression of GCCase protein from viral genomes may be determined using various methods known in the art such as, but not limited to immunochemistry (*e.g.*, IHC), enzyme-linked immunosorbent assay (ELISA), affinity ELISA, ELISPOT, flow cytometry, immunocytology, surface plasmon resonance analysis, kinetic exclusion assay, liquid chromatography-mass spectrometry (LCMS), high-performance liquid chromatography (HPLC), BCA assay, immunoelectrophoresis, Western blot, SDS-PAGE, protein immunoprecipitation, PCR, and/or in situ hybridization (ISH). In some embodiments, transgenes encoding GCCase protein delivered in different AAV capsids may have different expression levels in different CNS tissues.

[0528] In certain embodiments, the GCCase protein is detectable by Western blot.

[0529] Alternatively methods of detecting GBA1 expression are known, including, for example, use of the methods and compounds as described in Int’l Pub. No. WO2019136484, incorporated herein by reference in its entirety.

VII. Kits and Devices

Kits

[0530] In some aspects, the present disclosure provides a variety of kits for conveniently

and/or effectively carrying out methods of the present disclosure. Typically, kits will comprise sufficient amounts and/or numbers of components to allow a user to perform multiple treatments of a subject(s) and/or to perform multiple experiments.

[0531] Any of the vectors, constructs, or GCase proteins of the present disclosure may be comprised in a kit. In some embodiments, kits may further include reagents and/or instructions for creating and/or synthesizing compounds and/or compositions of the present disclosure. In some embodiments, kits may also include one or more buffers. In some embodiments, kits of the disclosure may include components for making protein or nucleic acid arrays or libraries and thus, may include, for example, solid supports.

[0532] In some embodiments, kit components may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and suitably aliquoted. Where there is more than one kit component, (labeling reagent and label may be packaged together), kits may also generally contain second, third or other additional containers into which additional components may be separately placed. In some embodiments, kits may also comprise second container means for containing sterile, pharmaceutically acceptable buffers and/or other diluents. In some embodiments, various combinations of components may be comprised in one or more vial. Kits of the present disclosure may also typically include means for containing compounds and/or compositions of the present disclosure, *e.g.*, proteins, nucleic acids, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which desired vials are retained.

[0533] In some embodiments, kit components are provided in one and/or more liquid solutions. In some embodiments, liquid solutions are aqueous solutions, with sterile aqueous solutions being particularly used. In some embodiments, kit components may be provided as dried powder(s). When reagents and/or components are provided as dry powders, such powders may be reconstituted by the addition of suitable volumes of solvent. In some embodiments, it is envisioned that solvents may also be provided in another container means. In some embodiments, labeling dyes are provided as dried powders. In some embodiments, it is contemplated that 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500, 600, 700, 800, 900, 1000 micrograms or at least or at most those amounts of dried dye are provided in kits of the disclosure. In such embodiments, dye may then be resuspended in any suitable solvent, such as DMSO.

[0534] In some embodiments, kits may include instructions for employing kit components as

well the use of any other reagent not included in the kit. Instructions may include variations that may be implemented.

Devices

[0535] In some embodiments, compounds and/or compositions of the present disclosure may be combined with, coated onto or embedded in a device. Devices may include, but are not limited to, dental implants, stents, bone replacements, artificial joints, valves, pacemakers and/or other implantable therapeutic device.

[0536] The present disclosure provides for devices which may incorporate viral vectors that encode one or more GCa6 protein molecules. These devices contain in a stable formulation the viral vectors which may be immediately delivered to a subject in need thereof, such as a human patient.

[0537] Devices for administration may be employed to deliver the viral vectors encoding GCa6 protein of the present disclosure according to single, multi- or split-dosing regimens taught herein.

[0538] Method and devices known in the art for multi-administration to cells, organs and tissues are contemplated for use in conjunction with the methods and compositions disclosed herein as embodiments of the present disclosure.

VIII. Definitions

[0539] At various places in the present specification, substituents of compounds of the present disclosure are disclosed in groups or in ranges. It is specifically intended that the present disclosure include each and every individual sub-combination of the members of such groups and ranges. The following is a non-limiting list of term definitions.

[0540] *Adeno-associated virus*: As used herein, the term “adeno-associated virus” or “AAV” refers to members of the dependovirus genus or a variant, e.g., a functional variant, thereof. In some embodiments, the AAV is wildtype, or naturally occurring. In some embodiments, the AAV is recombinant.

[0541] *AAV Particle*: As used herein, an “AAV particle” refers to a particle or a virion comprising an AAV capsid, e.g., an AAV capsid variant, and a polynucleotide, e.g., a viral genome or a vector genome. In some embodiments, the viral genome of the AAV particle comprises at least one payload region and at least one ITR. In some embodiments, an AAV particle of the disclosure is an AAV particle comprising an AAV capsid polypeptide, e.g., a parent capsid sequence with at least one peptide insert. In some embodiments, the AAV particle is capable of delivering a nucleic acid, e.g., a payload region, encoding a payload to cells, typically, mammalian, e.g., human, cells. In some embodiments, an AAV particle of the present

disclosure may be produced recombinantly. In some embodiments, an AAV particle may be derived from any serotype, described herein or known in the art, including combinations of serotypes (e.g., “pseudotyped” AAV) or from various genomes (e.g., single stranded or self-complementary). In some embodiments, the AAV particle may be replication defective and/or targeted. In some embodiments, the AAV particle may comprises a peptide present, e.g., inserted into, the capsid to enhance tropism for a desired target tissue. It is to be understood that reference to the AAV particle of the disclosure also includes pharmaceutical compositions thereof, even if not explicitly recited.

[0542] *Amelioration*: As used herein, the term “amelioration” or “ameliorating” refers to a lessening of severity of at least one indicator of a condition or disease. For example, in the context of a neurodegenerative disorder, amelioration includes the reduction or stabilization of neuron loss.

[0543] *Approximately*: As used herein, the term “approximately” or “about,” as applied to one or more values of interest, refers to a value that is similar to, i.e., within 10% of, a stated reference value.

[0544] *Capsid*: As used herein, the term “capsid” refers to the exterior, e.g., a protein shell, of a virus particle, e.g., an AAV particle, that is substantially (e.g., >50%, >60%, >70%, >80%, >90%, >95%, >99%, or 100%) protein. In some embodiments, the capsid is an AAV capsid comprising an AAV capsid protein described herein, e.g., a VP1, VP2, and/or VP3 polypeptide. The AAV capsid protein can be a wild-type AAV capsid protein or a variant, e.g., a structural and/or functional variant from a wild-type or a reference capsid protein, referred to herein as an “AAV capsid variant.” In some embodiments, the AAV capsid variant described herein has the ability to enclose, e.g., encapsulate, a viral genome and/or is capable of entry into a cell, e.g., a mammalian cell. In some embodiments, the AAV capsid variant described herein may have modified tropism compared to that of a wild-type AAV capsid, e.g., the corresponding wild-type capsid.

[0545] *Central Nervous System or CNS*: As used herein, “central nervous system” or “CNS” refers to one of the two major subdivisions of the nervous system, which in vertebrates includes the brain and spinal cord. The central nervous system coordinates the activity of the entire nervous system.

[0546] *Cervical Region*: As used herein, “cervical region” refers to the region of the spinal cord comprising the cervical vertebrae C1, C2, C3, C4, C5, C6, C7, and C8.

[0547] *Cis-Elements*: As used herein, cis-elements or the synonymous term “cis-regulatory elements” refer to regions of non-coding DNA which regulate the transcription of nearby genes.

The Latin prefix “cis” translates to “on this side.” Cis-elements are found in the vicinity of the gene, or genes, they regulate. Examples of cis-elements include a Kozak sequence, SV40 introns, or a portion of the backbone.

[0548] *CNS tissue:* As used herein, “CNS tissue” or “CNS tissues” refers to the tissues of the central nervous system, which in vertebrates, include the brain and spinal cord and sub-structures thereof.

[0549] *CNS structures:* As used herein, “CNS structures” refers to structures of the central nervous system and sub-structures thereof. Non-limiting examples of structures in the spinal cord may include, ventral horn, dorsal horn, white matter, and nervous system pathways or nuclei within. Non-limiting examples of structures in the brain include, forebrain, midbrain, hindbrain, diencephalon, telencephalon, myelencephalon, metencephalon, mesencephalon, prosencephalon, rhombencephalon, cortices, frontal lobe, parietal lobe, temporal lobe, occipital lobe, cerebrum, thalamus, hypothalamus, tectum, tegmentum, cerebellum, pons, medulla, amygdala, hippocampus, basal ganglia, corpus callosum, pituitary gland, putamen, striatum, ventricles and sub-structures thereof.

[0550] *CNS Cells:* As used herein, “CNS cells” refers to cells of the central nervous system and sub-structures thereof. Non-limiting examples of CNS cells include, neurons and sub-types thereof, glia, microglia, oligodendrocytes, ependymal cells and astrocytes. Non-limiting examples of neurons include sensory neurons, motor neurons, interneurons, unipolar cells, bipolar cells, multipolar cells, pseudounipolar cells, pyramidal cells, basket cells, stellate cells, Purkinje cells, Betz cells, amacrine cells, granule cell, ovoid cell, medium aspiny neurons and large aspiny neurons.

[0551] *Codon optimization:* As used herein, the term “codon optimization” refers to a process of changing codons of a given gene in such a manner that the polypeptide sequence encoded by the gene remains the same.

[0552] *Conservative amino acid substitution:* As used herein, a "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

[0553] *Derivative*: As used herein, the term “derivative” refers to a composition (*e.g.*, sequence, compound, formulation, *etc.*) that is derived from, or finds its basis in, a parent composition. Non-limiting examples of a parent composition include a wild-type or original amino acid or nucleic acid sequence, or an undiluted formulation. In some embodiments, a derivative is a variant of a parent composition. A derivative may differ from the parent composition by less than about 1%, less than about 5%, less than about 10%, less than about 15%, less than about 20%, less than about 25%, less than about 30%, less than about 35%, less than about 40%, less than about 45%, or less than about 50%. In certain embodiments, a derivative may differ from a parent composition by more than about 50%. In certain embodiments, a derivative may differ from a parent composition by more than about 75%. In some embodiments, a derivative may be a fragment or truncation of a parent amino acid or nucleotide sequence. As a non-limiting example, a derivative may be a sequence with a nucleotide or peptide insert as compared to a parent nucleic acid or amino acid sequence (*e.g.*, AAVPHP.B as compared to AAV9).

[0554] *Effective amount*: As used herein, the term “effective amount” of an agent is that amount sufficient to effect beneficial or desired results, for example, upon single or multiple dose administration to a subject or a cell, in curing, alleviating, relieving or improving one or more symptoms of a disorder and, as such, an “effective amount” depends upon the context in which it is being applied. For example, in the context of administering an agent that treats Parkinson Disease (PD) and related disorders, including Gaucher Disease, and Dementia with Lewy Bodies (collectively, “GBA-related disorders”), an effective amount of an agent is, for example, an amount sufficient to achieve treatment, as defined herein, of a GBA-related disorder as compared to the response obtained without administration of the agent.

[0555] *Excipient*: As used herein, the term “excipient” refers to an inactive substance that serves as the vehicle or medium for an active pharmaceutical agent or other active substance.

[0556] *Fragment*: A “fragment,” as used herein, refers to a contiguous portion of a reference sequence that retains at least one activity of the reference sequence. For example, fragments of proteins may comprise polypeptides obtained by digesting full-length protein isolated from cultured cells. A fragment may also refer to a truncation (*e.g.*, an N-terminal and/or C-terminal truncation) of a protein or a truncation (*e.g.*, at the 5' and/or 3' end) of a nucleic acid. A protein fragment may be obtained by expression of a truncated nucleic acid, such that the nucleic acid encodes a portion of the full-length protein.

[0557] *GBA/Gcase protein*: As used herein, the terms “Gcase”, “Gcase protein,” “GBA protein”, and “GBA1 protein” are used interchangeably to refer to a protein product or a

functional portion thereof of the GBA1 gene (Ensemble gene ID: ENSG00000177628).

[0558] *Humanized*: As used herein, the term “humanized” refers to a non-human sequence of a polynucleotide or a polypeptide which has been altered to increase its similarity to a corresponding human sequence.

[0559] *Identity*: As used herein, the term “identity” refers to the overall relatedness between polymeric molecules, *e.g.*, between oligonucleotide molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of the percent identity of two polynucleotide sequences, for example, may be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using methods such as those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; each of which is incorporated herein by reference in its entirety. For example, the percent identity between two nucleotide sequences can be determined, for example using the algorithm of Meyers and Miller (CABIOS, 1989, 4:11-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix. Methods commonly employed to determine percent identity between sequences include, but are not limited to those disclosed in Carillo, H., and Lipman, D., SIAM J Applied Math., 48:1073 (1988); incorporated herein by reference in its entirety. Techniques for determining identity are codified in publicly available computer

programs. Computer software to determine homology between two sequences include, but are not limited to, GCG program package, Devereux, J., *et al.*, *Nucleic Acids Research*, 12(1), 387 (1984)), BLASTP, BLASTN, and FASTA Altschul, S. F. *et al.*, *J. Molecular Biol.*, 215, 403 (1990)).

[0560] *Isolated*: As used herein, the term “isolated” refers to a substance or entity that is altered or removed from the natural state, e.g., altered or removed from at least some of component with which it is associated in the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not “isolated,” but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is “isolated.” An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of the environment in which it is found in nature. In some embodiments, an isolated nucleic acid is recombinant, e.g., incorporated into a vector.

[0561] *Lumbar Region*: As used herein, the term “lumbar region” refers to the region of the spinal cord comprising the lumbar vertebrae L1, L2, L3, L4, and L5.

[0562] *miR binding site*: As used herein, the “miR binding site” refers either to a DNA sequence corresponding to an RNA sequence that is bound by a microRNA, or to the RNA sequence that is bound by the microRNA.

[0563] *Operably linked*: As used herein, the phrase “operably linked” refers to a functional connection between two or more molecules, constructs, transcripts, entities, moieties, or the like.

[0564] *Payload*: As used herein, “payload” or “payload region” refers to one or more polynucleotides or polynucleotide regions encoded by or within a viral genome or an expression product of such polynucleotide or polynucleotide region, e.g., a transgene, a polynucleotide encoding a polypeptide.

[0565] *Payload construct*: As used herein, “payload construct” is one or more polynucleotide regions encoding or comprising a payload that is flanked on one or both sides by an inverted terminal repeat (ITR) sequence. The payload construct is a template that is replicated in a viral production cell to produce a viral genome.

[0566] *Payload construct vector*: As used herein, “payload construct vector” is a vector encoding or comprising a payload construct, and regulatory regions for replication and expression in bacterial cells. The payload construct vector may also comprise a component for viral expression in a viral replication cell.

[0567] *Pharmaceutically acceptable*: The phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are suitable for use in contact with the tissues of human beings and animals.

[0568] *Pharmaceutically acceptable excipients*: As used herein, the term “pharmaceutically acceptable excipient,” as used herein, refers to any ingredient other than active agents (*e.g.*, as described herein) present in pharmaceutical compositions that can function as vehicles for suspending and/or dissolving active agents.

[0569] *Pharmaceutically acceptable salts*: Pharmaceutically acceptable salts of the compounds described herein are forms of the disclosed compounds wherein the acid or base moiety is in its salt form (*e.g.*, as generated by reacting a free base group with a suitable organic acid). Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like.

[0570] *Pharmaceutical Composition*: As used herein, the term “pharmaceutical composition” or pharmaceutically acceptable composition” comprises AAV polynucleotides, AAV genomes, or AAV particle and one or more pharmaceutically acceptable excipients, solvents, adjuvants, and/or the like.

[0571] *Preventing*: As used herein, the term “preventing” refers to partially or completely delaying onset of an infection, disease, disorder and/or condition; partially or completely delaying onset of one or more symptoms, features, or clinical manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying onset of one or more symptoms, features, or manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying progression from an infection, a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the infection, the disease, disorder, and/or condition.

[0572] *Purified*: As used herein, the term “purify” means to make substantially pure or clear from one or more unwanted components, material defilement, admixture or imperfection. “Purified” refers to the state of being pure. “Purification” refers to the process of making pure. As used herein, a substance is “pure” if it is substantially free of (substantially isolated from) one or more components, *e.g.*, one or more components found in a native context.

[0573] *Region*: As used herein, the term “region” refers to a zone or general area. In some embodiments, when referring to a protein or protein module, a region may comprise a linear sequence of amino acids along the protein or protein module or may comprise a three dimensional area, an epitope and/or a cluster of epitopes. In some embodiments, regions

comprise terminal regions. As used herein, the term “terminal region” refers to regions located at the ends or termini of a given agent. When referring to proteins, terminal regions may comprise N- and/or C-termini. N-termini refer to the end of a protein comprising an amino acid with a free amino group. C-termini refer to the end of a protein comprising an amino acid with a free carboxyl group. N- and/or C-terminal regions may comprise the N- and/or C-termini as well as surrounding amino acids.

[0574] *Sample:* As used herein, the term “sample” refers to an aliquot or portion taken from a source and/or provided for analysis or processing. In some embodiments, a sample is from a biological source such as a tissue, cell or component part (*e.g.* a body fluid, including but not limited to blood, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen).

[0575] *Serotype:* As used herein, the term “serotype” refers to distinct variations in a capsid of an AAV based on surface antigens which allow epidemiologic classifications of the AAVs at the sub-species level.

[0576] *Signal Sequences:* As used herein, the phrase “signal sequences” refers to a sequence which can direct the transport or localization.

[0577] *Similarity:* As used herein, the term “similarity” refers to the overall relatedness between polymeric molecules, *e.g.*, between polynucleotide molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of percent similarity of polymeric molecules to one another can be performed in the same manner as a calculation of percent identity, except that calculation of percent similarity takes into account conservative substitutions as is understood in the art.

[0578] *Spacer:* As used herein, a “spacer” is generally any selected nucleic acid sequence of, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length, which is located between two or more consecutive miR binding site sequences.

[0579] *Subject:* As used herein, the term “subject” or “patient” refers to any organism to which a composition in accordance with the disclosure may be administered, *e.g.*, for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Similarly, “subject” or “patient” refers to an organism who may seek, who may require, who is receiving, or who will receive treatment or who is under care by a trained professional for a particular disease or condition. Typical subjects include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and humans). In certain embodiments, a subject or patient may be susceptible to or suspected of having a GBA-related disorder. In certain embodiments, a subject or patient may be diagnosed with PD, Gaucher Disease, or Dementia with Lewy Bodies disease.

[0580] *Substantially*: As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0581] *Suffering from*: An individual who is “suffering from” a disease, disorder, and/or condition has been diagnosed with or displays one or more symptoms of a disease, disorder, and/or condition.

[0582] *Susceptible to*: An individual who is “susceptible to” a disease, disorder, and/or condition has not been diagnosed with and/or may not exhibit symptoms of the disease, disorder, and/or condition but harbors a propensity to develop a disease or its symptoms. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition (for example, cancer) may be characterized by one or more of the following: (1) a genetic mutation associated with development of the disease, disorder, and/or condition; (2) a genetic polymorphism associated with development of the disease, disorder, and/or condition; (3) increased and/or decreased expression and/or activity of a protein and/or nucleic acid associated with the disease, disorder, and/or condition; (4) habits and/or lifestyles associated with development of the disease, disorder, and/or condition; (5) a family history of the disease, disorder, and/or condition; and (6) exposure to and/or infection with a microbe associated with development of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[0583] *Therapeutic Agent*: The term “therapeutic agent” refers to any agent that, when administered to a subject has a therapeutic, diagnostic, and/or prophylactic effect and/or elicits a desired biological and/or pharmacological effect.

[0584] *Therapeutically effective amount*: As used herein, the term “therapeutically effective amount” means an amount of an agent to be delivered (*e.g.*, nucleic acid, drug, therapeutic agent, diagnostic agent, prophylactic agent, *etc.*) that is sufficient, when administered to a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, delay progression of symptoms, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is provided in a single dose. In some embodiments, a therapeutically effective

amount is administered in a dosage regimen comprising a plurality of doses. Those skilled in the art will appreciate that in some embodiments, a unit dosage form may be considered to comprise a therapeutically effective amount of a particular agent or entity if it comprises an amount that is effective when administered as part of such a dosage regimen.

[0585] *Thoracic Region:* As used herein, a “thoracic region” refers to a region of the spinal cord comprising the thoracic vertebrae T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, and T12.

[0586] *Treating:* As used herein, the term “treating” refers to partially or completely alleviating, ameliorating, improving, relieving, reversing, delaying onset of, inhibiting progression of, reducing severity of, and/or reducing incidence of one or more symptoms or features of a particular infection, disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition and/or to a subject who exhibits only early signs of a disease, disorder, and/or condition for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[0587] *Unmodified:* As used herein, “unmodified” refers to any substance, compound or molecule prior to being changed in any way. Unmodified may, but does not always, refer to the wild-type or native form of a biomolecule or entity. Molecules or entities may undergo a series of modifications whereby each modified product may serve as the “unmodified” starting molecule or entity for a subsequent modification.

[0588] *Variant:* The term “variant” refers to a polypeptide or polynucleotide that has an amino acid or a nucleotide sequence that is substantially identical, e.g., having at least 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to a reference sequence. In some embodiments, the variant is a functional variant. As used herein, the term “functional variant” refers to a polypeptide variant or a polynucleotide variant that has at least one activity of the reference sequence.

[0589] *Vector:* As used herein, a “vector” is any molecule or moiety which transports, transduces or otherwise acts as a carrier of a heterologous molecule. Vectors of the present disclosure may be produced recombinantly and may be based on and/or may comprise adeno-associated virus (AAV) parent or reference sequence(s). Such parent or reference AAV sequences may serve as an original, second, third or subsequent sequence for engineering vectors. In non-limiting examples, such parent or reference AAV sequences may comprise any one or more of the following sequences: a polynucleotide sequence encoding a polypeptide or multi-polypeptide, having a sequence that may be wild-type or modified from wild-type and

which sequence may encode full-length or partial sequence of a protein, protein domain, or one or more subunits of GCase protein and variants thereof; a polynucleotide encoding GCase protein and variants thereof, having a sequence that may be wild-type or modified from wild-type; and a transgene encoding GCase protein and variants thereof that may or may not be modified from wild-type sequence.

[0590] *Viral genome:* As used herein, a “viral genome” or “vector genome” is a polynucleotide comprising at least one inverted terminal repeat (ITR) and at least one encoded payload. A viral genome encodes at least one copy of the payload.

EXAMPLES

[0591] The present disclosure is further illustrated by the following non-limiting examples. Experiments described in the Examples establish that enhanced AAV-based GCase gene therapy treatments are superior to and/or additive with wild-type GCase-based treatment in ameliorating GBA1-related disorders.

Cell Lines, Tissues, and Animal Models

[0592] *In vitro* experiments: Human fibroblasts from GBA1 patients (all 3 types) were obtained from Coriell. The following Gaucher patient fibroblasts were chosen based on significantly depleted GCase activity (4-6%) and availability of age- and race-matched healthy control fibroblasts: GM04394-fibroblast, GM00852-fibroblast, GM00877-fibroblast, GM05758-fibroblast from skin/inguinal area, and GM02937-fibroblast from skin/unspecified (all available from Coriell).

[0593] GBA1-4L/PS-NA primary neurons can be generated from pregnant GBA1-4L/PS-NA females from QPS. GBA1-knockout (GBA1-KO) neuroblastoma cell line (IMR-32 background, available from ATCC) was obtained from Synthego.

[0594] Animal models: GBA1-4L/PS-NA mouse models (available at QPS): 4L/PS-NA mice express low level of prosaposin and saposin C, as well as GCase with a point mutation at position V394L/V394L. Strong enlargement of leukocytes and macrophages in visceral organs like spleen, thymus, lung and liver develop as early as 5 week of age. Most deficits and reduced muscle strength accompanied by neuroinflammation in the cortex, and hippocampus increase as animals age. There is significant increase in glucosylceramide and glucosylsphingosine. GBA1-4L/PS-NA can survival up to 22 weeks. Homozygous Prnp-SNCA-A53T (M83) mice, by 8-months of age, develop α -syn aggregates and progressively severe motor phenotype.

Example 1. Vector Design and Synthesis

[0595] An AAV viral genome expressing a payload region comprising a polynucleotide encoding a human GBA1 polypeptide is generated. The viral genome comprises

polynucleotides encoding an AAV capsid of a serotype provided in Table 1. A promoter region regulates expression of the payload region. Widespread GBA1 distribution is achieved by use of a ubiquitous promoter, such as CBA, to achieve transduction within different CNS cell types.

[0596] Single-stranded codon optimized GBA1 cDNA sequence under ubiquitous CBA promoter packaged within AAV2 ITRs is generated (wtGBA). Enhanced GBA1 (enGBA) constructs (see Examples 2-5) are generated and compared against wtGBA. wtGBA1 and GFP reporter vectors are compared side-by-side to test multiplicity of infection for *in vitro* experiments. The final AAV transgene design nominations are made based on vectorized *in vitro* experiments and tested in the proposed *in vivo* models, including those used for GLP and tolerability studies.

[0597] PD-GBA1 patients demonstrate a global reduction in GCase levels in the CNS. Consequently, high GCase levels in CSF, caudate, substantia nigra, cortex and cerebellum is targeted. Although the disease pathology is largely neuronal, the therapeutic strategy is expected to benefit by transduction of other CNS cell-types, *e.g.* astrocytes, via cross-correction benefit.

[0598] In addition to PD-GBA, efficacy in secondary disease indications in patients with GBA1 mutations is tested, including Gaucher disease (including Neuronopathic Gaucher disease) and Dementia with Lewy bodies.

[0599] Transgenes designed as described above are tested for plasmid-level expression: all cassettes are engineered in single stranded AAV transgene configuration driven by ubiquitous CBA promoter flanked by AAV2 ITRs. The following transgene constructs are engineered and synthesized: 1) codon optimized GBA1 cDNA construct; 2) enhanced GBA1 construct comprising GBA1 cDNA and further encoding prosaposin/saposin C in the same transgene (optimal co-activator gene and linker sequences are selected for vectorization based on plasmid-level expression analysis); 3) enhanced GBA1 constructs comprising a cell-penetration peptide; and 4) enhanced GBA1 constructs comprising lysosomal targeting peptides (LTP); and 5) combinatorial enhanced GBA1 constructs comprising a combination of GBA1 cDNA, saposin sequence(s), lysosomal targeting sequence(s) and/or cell penetrating peptide sequence(s).

[0600] These constructs are tested for expression/GCase activity in cell culture with ITR plasmid transfections as a first pass. Specifically, plasmids are tested in CHO/HEK-293 cells at 48 hours post transfections. Both lysates and media are assessed for expression. Based on the results, GBA1 transgene ITR cassettes (wt, enGBA1 and enGBAcombo constructs) are selected for vectorization and evaluation within *in vitro* disease model setting.

Upon plasmid-level expression/GCase activity confirmation, select AAV ITR cassettes (wtGBA, enGBA1 and enGBAcombo) are packaged into HEK 293 small-scale AAV6 or AAV2 preps for

initial *in vitro* evaluations.

Example 2. Co-administration of SapC enhances GBA1 gene therapy

[0601] Viral genomes encoding a GBA1 protein can also be designed to further encode an enhancement element, e.g., a prosaposin protein, a Saposin C protein, or functional variant thereof. GCase coactivator Saposin C (SapC) is one of the cleavage products of saposin precursor protein Prosaposin. Saposin C is the essential activator of GCase lysosomal enzyme. In mouse models of PD-GBA1 and Gaucher disease, the combination of loss of function in GBA1 and Saposin C results in significantly exacerbated disease phenotype. Thus, AAV mediated co-delivery of GBA1 (e.g., a viral genome encoding a GBA1 protein, e.g., comprising the nucleotide sequence of SEQ ID NO: 1772, 1773, 1776, 1777, 1780, or 1781, or a functional variant thereof) and cDNA encoding a prosaposin protein (e.g., a prosaposin protein comprising the amino acid sequence of SEQ ID NO: 1750 or 1758, or a functional variant thereof; or encoded by a nucleotide sequence comprising SEQ ID NO: 1858 or 1859, or a functional variant thereof) or a Saposin C (SapC) protein or functional variant thereof (e.g., a SapC protein or functional variant thereof comprising the amino acid sequence of SEQ ID NO: 1788, 1789, 1791, or 1792; or encoded by the nucleotide sequence of SEQ ID NO: 1786, 1787, 1790, or 1791) is tested to increase potency of GBA1 gene therapy by enhancing catalytic activity of GCase enzyme.

Example 3. Cell-penetration peptides enhance cellular penetration/uptake

[0602] Viral genomes encoding a GBA1 protein can also be designed to further encode an enhancement element, e.g., a cell penetrating peptide or functional variant thereof. Without wishing to be bound by theory, it is believed that a cell-penetration peptide (CPP) signal added to the GCase sequence of the transgenes of the disclosure results in increased cellular uptake of secreted GCase product in circulation, in cerebrospinal fluid, and in interstitial fluid from AAV-transduced cells; and this enhanced cell penetration thus increases cross-correction potential of the secreted GCase enzyme. Exemplary CPPs used herein include: HIV-derived TAT peptide (e.g., comprising the amino acid sequence of 1794 and/or encoded by the nucleotide sequence of SEQ ID NO: 1794), human apolipoprotein B receptor binding domain (e.g., comprising the amino acid sequence of 1796 and/or encoded by the nucleotide sequence of SEQ ID NO: 1795), and/or human apolipoprotein E-II receptor binding domain (e.g., comprising the amino acid sequence of 1798 and/or encoded by the nucleotide sequence of SEQ ID NO: 1797).

Example 4. CMA recognition sequences enhance intracellular lysosomal targeting

[0603] Viral genomes encoding a GBA1 protein can also be designed to further encode an enhancement element, e.g., a lysosomal targeting sequence or functional variant thereof.

[0604] Chaperone sequences including glycosylation-independent lysosomal targeting peptides (which is an M-6-P independent lysosomal targeting mechanism) have demonstrated ability to enable enhanced delivery of lysosomal enzyme product. GBA1 utilizes LIMP-2 (encoded by SCARB2 gene) as the lysosomal surface receptor (important for lysosomal localization). Co-delivery of SCARB2 with GBA1 provides an alternative strategy to enhance lysosomal targeting of GCase. Chaperone-mediated autophagy signals are incorporated into transgenes of the disclosure to increase lysosomal targeting of the GCase enzyme. Highly conserved recognition sequences of chaperone-mediated-autophagy (CMA) pathway is analyzed for improved lysosomal targeting of GCase enzyme. Such sequences include, for example, RNase A-derived CMA recognition sequence, HSC70-derived CMA recognition sequence, or hemoglobin-derived CMA recognition sequence.

[0605] Lysosomal targeting sequences (LTS) are also included in viral genomes encoding a GBA1 protein described herein. Exemplary LTS peptides used herein include LTS1 (e.g., comprising the amino acid sequence of SEQ ID NO 1800 and/or encoded by the nucleotide sequence of SEQ ID NO: 1799), LTS2 (e.g., comprising the amino acid sequence of SEQ ID NO 1802 and/or encoded by the nucleotide sequence of SEQ ID NO: 1801), LTS3 (e.g., comprising the amino acid sequence of SEQ ID NO 1804 and/or encoded by the nucleotide sequence of SEQ ID NO: 1803), LTS4 (e.g., comprising the amino acid sequence of SEQ ID NO 1806 and/or encoded by the nucleotide sequence of SEQ ID NO: 1805), and/or LTS5 (e.g., comprising the amino acid sequence of SEQ ID NO 1808 and/or encoded by the nucleotide sequence of SEQ ID NO: 1807).

Example 5. Combinatorial enhancements

[0606] Combinations of the aforementioned enhancement elements (Examples 2-4) are tested for ability of different combinations to additively or synergistically increase potency of AAV mediated delivery of GCase enzyme *in vivo* (by various possible combinations of enhanced cross-correction, enhanced lysosomal targeting, enhanced catalytic activity). These combinatorial approaches are also compared against a reference transgene (SEQ ID NO: 1759) for various aforementioned outcomes. Without wishing to be bound by theory, it is believed that transgene-level enhancements can increase the potency of AAV gene therapy and reduce the minimal efficacious dose for *in vivo* evaluations and clinic applications.

Example 6. *In vitro* Screen

[0607] Early *in vitro* experiments are designed to enable validation of the functional enhancements made in the GBA1 transgene by conducting side-by-side comparison against a reference GBA1 construct (e.g., SEQ ID NO: 1759). Experiments are run as AAV vectorized

transduction studies on *in vitro* models of GBA1 LOF (patient fibroblasts or GBA1 knockout mouse primary neurons). Dose response is determined. Post-translational modifications and activity of the final GCase product can also be determined.

[0608] *In vitro* evaluations with AAV vectors packaging wtGBA, enGBA1 and enGBAcombo are carried out on patient fibroblasts with GBA1 mutations and primary neurons derived from WT and/or 4L/PS-NA GBA1 mouse model. In preparation for generating 3-point dose-response curves of all AAV.GBA1 vectors generated, AAV6.CBA.Luciferase reporter-gene transduction assay is performed on the 2 cell lines to verify optimal experimental conditions, *e.g.* Multiplicity Of Infection (MOI) for AAV6 vectors to be applied across *in vitro* screening. Once the *in vitro* experiments are optimized, head-to-head comparisons to identify optimal enGBA1 transgene configurations for further *in vivo* evaluations can be conducted.

[0609] *In vitro* dose-response comparisons of AAV-enGBA1 or AAV-wtGBA1 constructs (*e.g.*, any one of SEQ ID NOs: 1759-1771 or 1809-1828, *e.g.*, as described in Tables 18-21 or 29-32) for GCase activity: To determine if GBA1 transgene enhancement strategies confer increased GCase activity in disease-relevant *in vitro* models, human fibroblasts with and without GBA1 mutations, and WT/GBA1 mutant mouse primary neurons are treated with AAV6 vectors packaging enhanced GBA1 viral genome variants at 3 different MOIs. At terminal time point, secreted and intracellular GCase expression and activity are measured in both media and cell lysate. Additionally, ddPCR based vector genome analysis is conducted to ensure successful *in vitro* gene transfer across different conditions.

[0610] *In vitro* comparison of AAV-enGBA1 constructs for subcellular localization: Whether the GBA1 transgene enhancement strategies confer increased lysosomal localization properties in healthy and disease relevant *in vitro* models is determined. Human fibroblasts with/without GBA1 mutations; and WT/GBA1 mutant mouse primary neurons are treated with AAV6 vectors packaging enhanced GBA1 viral genome variants. At terminal time point, cells are fixed and co-immunostained for HA (AAV transduction) and lysosomal markers (*e.g.* Lamp1). Transduction efficiency and % colocalization in the lysosomes are assessed for all AAV vectors using the Bio-Tek Cytation 5 for image analysis and quantification.

[0611] *In vitro* comparison of AAV-enGBA1 constructs for enzyme cross-correction: In addition to intracellular and secreted GCase activity, AAV-GBA1 transgene enhancement strategies are assessed for cross-correction properties in disease relevant *in vitro* conditions. Non-GBA/GBA1 human fibroblasts and GBA1 mutant/WT mouse primary neurons are treated with AAV6 vectors packaging enhanced GBA1 viral genomes. Conditioned media from transduced cells is collected at 24-, 48- and 72-hours post AAV treatments. In order to

recapitulate *in vivo* cross-correction, untreated human fibroblasts and mouse primary neurons are then treated with different conditioned media for 24 hours. At this point, a subset of wells is co-immunostained for HA (visualization of cross-corrected GCase protein product) and lysosomal markers (*e.g.* Lamp1). Another subset of wells is lysed and evaluated for GCase activity. Cross-correction efficiency and % colocalization in the lysosomes is visualized and quantified for all AAV vector treatments.

[0612] *In vitro* GBA1 MOI dose-response study with AAVwtGBA1 and AAVenGBA1 vectors: Small-scale preps of optimal AAV capsid packaging wtGBA1 and enGBA1 constructs. A 3-point dose-response infection study is conducted with wtGBA1 and enGBA1 packaging AAVs. GBA1-KO neuroblastoma cells (wtGBA1 neuroblastoma control) and Gaucher disease patient fibroblasts (healthy controls) are used for evaluations.

[0613] Select wtGBA1 or enGBA1 constructs are identified for further analysis *in vivo*.

Example 7. Assay development

[0614] One method of detecting GCase activity involves measuring turnover of an artificial substrate, 4-Methylumbelliferyl β -D-galactopyranoside (4-MUG) as described, for example in Rogers *et al.*, “Discovery, SAR, and biological evaluation of non-inhibitory chaperones of glucocerebrosidase.” (2010), incorporated herein by reference in its entirety. The 4-MUG assay is used to determine GCase activity and GCase concentration in cell lysates.

[0615] Another method of detecting GCase activity involves use of a SensoLyte Blue Glucocerebrosidase assay (AnaSpec, Fremont, CA), a fluorometric assay, according to the manufacturer’s instructions. Sensolyte Blue Glucocerebrosidase assay detects GCase activity using a fluorogenic analog-substrate, wherein the output is fluorescent excitation/emission at 365nm/445nm on a standard plate reader.

[0616] Fluorescence-based detection of hGBA1 in mouse tissue, and assessment of hGCase activity in mouse can be determined using the methods as described in Morabito, Giuseppe *et al.*, “AAV-PHP. B-mediated global-scale expression in the mouse nervous system enables GBA1 gene therapy for wide protection from synucleinopathy.” Molecular Therapy 25.12 (2017): 2727-2742, the contents of which are incorporated by reference herein in their entirety. Alternative methods of visualizing GCase activity are described, for example, in Chao, Daniela Herrera Moro *et al.*, “Visualization of active glucocerebrosidase in rodent brain with high spatial resolution following in situ labeling with fluorescent activity based probes.” PLoS One 10.9 (2015), the contents of which are incorporated herein by reference in their entirety. See also Witte, Martin D., *et al.* Nature Chemical Biology 6, 907-13 (2010), incorporated herein by reference in its entirety, describing “ultra-sensitive” cyclophellitol β -oxide (CBE) based probes

for highly specific GBA1 labeling *in vitro* and *in vivo*. CBE, a GCase inhibitor, irreversibly binds GBA1 and inhibits its GCase activity, has been shown to cross the blood-brain-barrier, and induces biochemical, clinical and histological manifestations of Gaucher disease (Kuo, Chi-Lin, *et al.* "In vivo inactivation of glycosidases by conduritol B epoxide and cyclophellitol as revealed by activity-based protein profiling." The FEBS journal 286.3 (2019): 584-600, incorporated herein by reference in its entirety).

[0617] For these assays, GCase/GBA1 protein concentration and activity are determined and normalized to total protein/activity levels in lysate. Negative control lysates are prepared from, for example, hippocampus and brainstem of vehicle (PBS) treated 6-8 week C57/Bl6 female mice (n=4). Positive control lysates are from human recombinant GBA1-infected/expressing cells. Inhibitor control lysates are from human recombinant GBA+GBA1-inhibitor infected/expressing cells to test specificity of the enzyme. An example GCase inhibitor for use in such studies is CBE. Vehicle/Lysis buffer/matrix controls consist of lysis buffer (Sigma) and substrate only. Background controls consist of substrate only. Minimal protein concentrations needed to observe GCase activity are identified by analysis of additional dilutions of lysate.

[0618] An assay is validated for evaluating increase in glucosylceramidase activity and glucosylceramidase protein concentration within *in vitro* GBA1 disease models (Gaucher patient fibroblasts and GBA1-KO neuroblastoma cells) post AAV administration.

Example 8. *In vivo* Screen

[0619] *In vivo* target engagement in GBA1 disease model: After *in vitro* screening, target engagement in a GBA1 mouse model (GBA1-4L/PS-NA) is demonstrated. A side-by-side comparison with AAV-wtGBA1 can be used to further bolster findings and demonstrate efficacy *in vivo*. *In vivo* evaluations determine whether AAV9-enGBA1 and AAV9-enGBAcombo candidate treatments selected during *in vitro* evaluations result in comparable/significantly higher GCase activity and reduction in GluCer and Glucosylsphingosine substrate-level reduction benefit as compared to AAV9-GBA1 reference construct in GBA1 a mouse model.

[0620] Up to 10 top enGBA1 constructs with significantly favorable attributes as compared to GBA1 reference construct using the GBA1 4L/PS-NA mouse model (available at QPS) are tested. AAV-untreated non transgenic (NT) mice are used as controls for biochemical analyses. GBA1-4L/PS-NA mice show relevant features of human GBA1 mutations including significantly reduced GCase activity and increased Glucosylceramide and Glucosylsphingosine as early as 5 weeks post birth. Neuroinflammation in the CTx and hippocampus is also seen in these mice.

[0621] In order to assess target engagement in the GBA1 disease model, intrastriatal

administration of AAV9 vectors packaging GBA1 reference construct and up to top 10 of the enhanced GBA1 variant viral genomes at three doses 5×10^9 , 1×10^{10} , 5×10^{10} vg/inj via bilateral injections is performed. Animals are euthanized 4 weeks post injections and CNS, peripheral tissues, and fluid compartments (serum and CSF) are collected for AAV biodistribution and transduction (GCase activities and GluCer substrate levels) analyses. Successful/lead candidates cause modest increase (~30% over baseline) of GCase activity in GBA1 animal models. Untreated strain- and age-matched WT mice are included to compare physiological levels of GCase and GluCer in healthy animals. Thus, intrastriatal enGBA/enGBAcombo treatments result in equivalent/superior physiological restoration of GCase enzyme levels in the CNS tissues and CSF of GBA1 mutant mice as compared to GBA1 reference construct. Concomitantly similar comparison is also made for Glucosylceramide or Glucosylsphingosine levels for different treatments for substrate reduction. Up to 3 top AAV9-enGBA1 treatments are advanced for efficacy studies.

Example 9. *In Vivo* Efficacy Evaluations

[0622] Dose selection: *In vivo* target engagement hits identified in Examples 2-5 are evaluated for GCase expression, target engagement, and efficacy based on readouts in murine disease models of GBA1-PD. For efficacy determining *in vivo* studies, both GBA1-4L/PS-NA and SNCA-A53T (M83) mice are used; WT animals are compared as controls. Both mouse models (GBA1-4L/PS-NA and M83), n=6-10 mice per group, receive bilateral intrastriatal injections of 5×10^9 , 1×10^{10} , 5×10^{10} vg/inj (or other appropriate concentration based on study results) of the top hits. Mice are euthanized 4 or 8 weeks post-dose. CNS and peripheral tissues and fluid samples including cortex, striatum, thalamus, brain stem, cerebellum, CSF, serum and liver are collected. GCase expression and activity and GluCer substrate levels are measured. Early immunohistochemical readouts using Iba1, GFAP, and H&E stains of mouse brain, spinal cord and liver are performed in order to confirm tolerability at various AAV doses.

[0623] Efficacy evaluations will determine whether AAV-enGBA1 candidate treatments result in efficacious and sustained increase in GCase activity in the brain resulting in reduction in GCase substrate within GBA1-4L/PS-NA mouse model. Based on dose selection studies, AAV vectors that are well-tolerated and showing >30% increase in GBA1 protein expression in relevant CNS tissues are further tested in a time-response study. Briefly, GBA1-4L/PS-NA mice (n=6-10) are injected with an intrastriatal injection of lead constructs (dose determined based on dose-selection study), multiple CNS and peripheral tissues and fluid compartments (serum and CSF) are collected at various time-points (e.g. 4, 8 and 12 weeks) and GCase expression and activities, substrate reduction in the CNS and periphery are quantified. Lysosomal localization

of the transduced GCase enzyme product are confirmed with immune-colocalization of AAV transduction with lysosomal marker.

[0624] Further evaluations will assess whether AAV-enGBA1 candidate treatments result in efficacious and sustained increase in GCase activity in the brain resulting in reduction of α -Syn pathology within GBA1/ α -Synuclein A53T mouse model. Based on dose selection study, AAV vectors that are well-tolerated and showing >30% increase in GBA1 protein expression in relevant CNS tissues are further tested in SNCA-A53T (M83) mouse model. M83 mice are known to start developing α -syn pathology at 6-7 months of age with progressive motor deficits. M83 mice (n=8-12) are injected with most efficacious constructs (Intrastriatal; dose according to study results) at ~6 months of age; and evaluated for GCase expression and activity, and α -syn pathology 3 months post administration. Previous studies have shown therapeutic benefit of AAV-GBA1 in reducing α -syn aggregates in SNCA transgenic mouse models. Here, in addition to GCase expression and activities, AAV-enGBA1 candidate treatments which result in physiological restoration of GCase enzyme levels (>30%) in the CNS tissue and CSF are evaluated for α -syn pathology reduction in A53T (M83) mice using immunohistochemical analyses.

Example 10. Natural History Study

[0625] In parallel with Stage 1 screening efforts, phenotypic, biochemical and immunohistochemical analysis were performed on 4L/PS-NA, 4L control, and wild-type mice to establish disease-relevant efficacy readouts and timelines.

[0626] GBA1 and Saposin C expression levels were determined in forebrain, midbrain, and hindbrain sections of the mice by LC-MS/MS, and were normalized to actin levels. Consistently across 5, 12, and 18 weeks of age, in all regions of the brain, 4L/PS-NA mice had lower GBA1 expression levels compared to that of wild-type mice and similar levels of GBA1 expression as 4L mice (Table 11). The brain of wild-type mice generally showed a trend of increased GBA1 expression in the hindbrain relative to the midbrain the forebrain (Table 11). Additionally, a decrease in GBA1 levels in the forebrain and midbrain was observed in the wild-type mice between 5 weeks and 12-18 weeks of age.

Table 11: Avg. GBA1 level (GBA/Actin) in GBA1-related disease mouse models

Animal Model	5 Weeks			12 Weeks			18 Weeks		
	Region of Brain			Region of Brain			Region of Brain		
	Fore-brain	Mid-brain	Hind-brain	Fore-brain	Mid-brain	Hind-brain	Fore-brain	Mid-brain	Hind-brain
4L/PS-NA	473.5	715.2	996.7	591.0	760.5	779.0	801.8	726.6	907.6
4L	687.5	676.8	939.8	614.5	764.9	702.8	512.3	691.8	1094.2

Control									
Wild-type	3463.2	3644.1	5377.4	2616.6	2902.2	4468.3	2551.3	3273.6	5129.8

[0627] Saposin C (SapC)/Actin levels in 4L/PS-NA mice were lower than those observed in the 4L or wild-type mice in the forebrain, midbrain, and hindbrain (Table 12). SapC levels increased in the brains of wild-type mice, with the highest level quantified at 18 weeks of age (Table 12).

Table 12: Avg. SapC level (SapC/Actin) in GBA1-related disease mouse models

Animal Model	5 Weeks			12 Weeks			18 Weeks		
	Region of Brain			Region of Brain			Region of Brain		
	Fore-brain	Mid-brain	Hind-brain	Fore-brain	Mid-brain	Hind-brain	Fore-brain	Mid-brain	Hind-brain
4L/PS-NA	232.1	289.6	430.6	315.8	432.1	523.3	227.5	552.8	591.4
4L Control	748.6	1102.9	1769.3	844.2	1486.5	1506.3	646.5	1123.6	1546.4
Wild-type	1500.3	2283.3	3087.9	1662.3	1971.0	2968.9	1656.3	2143.9	3253.1

[0628] At five-weeks, 12 weeks, and 18 weeks of age, GCase activity also was measured in forebrain, midbrain and hindbrain tissue sections of 4L/PS-NA (model having decreased GCase and prosaposin), 4L control (model having decreased GCase) and wild-type (normal GCase and prosaposin) mice (Table 13).

[0629] At 5 weeks of age, decreased GCase activity was confirmed in both the 4L/PS-NA and 4L control mice, with significant GCase deficits as compared to wild-type mice. GCase activity was not significantly different between the 4L/PS-NA and 4L control mice (Table 13).

Table 13: Avg. GCase activity [RFU per mL] in GBA1-related disease mouse models

Animal Model	5 Weeks			12 Weeks			18 Weeks		
	Region of Brain			Region of Brain			Region of Brain		
	Fore-brain	Mid-brain	Hind-brain	Fore-brain	Mid-brain	Hind-brain	Fore-brain	Mid-brain	Hind-brain
4L/PS-NA	4318.36	6448.92	5581.51	6343.1	6423.7	5386.7	4109.2	6466.4	5981.6
4L Control	4442.15	5614.69	5598.62	4969.0	4640.2	6798.4	5837.2	5915.6	8310.4
Wild-type	9531.29	10448.20	10520.70	8379.2	7596.1	9286.1	5488.1	9730.8	8227.2

[0630] Similarly, in 12 and 18 weeks old mice, decreased GCase activity was quantified in both the 4L/PS-NA and 4L control mice, with significant GCase deficits as compared to wild-type mice (Table 13). GCase activity was also not significantly different between the 4L/PS-NA

and 4L control mice at 12 and 18 weeks of age (Table 13).

[0631] Also, at five-weeks, 12 weeks, and 18 weeks of age, GBA1 substrate levels, specifically glucosylsphingosine (GlcSph) and glucosylceramide (GlcCer), were measured by LC-MS/MS in forebrain, midbrain and hindbrain tissue sections of 4L/PS-NA (model having decreased GCase and prosaposin), 4L control (model having decreased GCase) and wild-type (normal GCase and prosaposin) mice and normalized to actin. As shown in Table 14, the greatest increase in GlcSph levels was observed in the brains of the 4L/PS-NA mice followed by 4L-control mouse brains, relative to the wild-type mouse. Additionally, GlcSph levels in the 4L/PS-NA mouse brains and 4L control mouse brains increased with age and higher levels were observed in the hindbrain, as compared to the forebrain or midbrain. These data demonstrate the effects of reduced GCase activity and decreased GBA1 levels in these mice, as measured above.

Table 14: Avg. glucosylsphingosine level (GlcSph/Actin) in GBA1-related disease mouse models

Animal Model	5 Weeks			12 Weeks			18 Weeks		
	Region of Brain			Region of Brain			Region of Brain		
	Fore-brain	Mid-brain	Hind-brain	Fore-brain	Mid-brain	Hind-brain	Fore-brain	Mid-brain	Hind-brain
4L/PS-NA	1086.1	1050.9	1384.0	1792.6	1897.0	2543.6	2571.0	3113.1	5672.8
4L Control	268.0	317.0	308.7	404.5	435.2	520.1	465.8	453.4	455.1
Wild-type	0	0	0	0	0	0	0	0	0

[0632] As shown in Table 15, the levels of GlcCer was increased in the 4L/PS-NA mouse brains, and the levels were higher in the hindbrain as compared to the forebrain and the midbrain. Levels of GlcCer were also higher at 18 weeks of age in the 4L/PS-NA mouse brains. These data also support the effects of reduced GCase activity and decreased GBA1 levels in these mice, as measured above.

Table 15: Avg. glucosylceramide (GlcCer) 18:1/18:0/Actin in GBA1-related disease mouse models

Animal Model	5 Weeks			12 Weeks			18 Weeks		
	Region of Brain			Region of Brain			Region of Brain		
	Fore-brain	Mid-brain	Hind-brain	Fore-brain	Mid-brain	Hind-brain	Fore-brain	Mid-brain	Hind-brain
4L/PS-NA	2179.2	1875.6	2247.5	7894.8	6425.0	12123.0	14774.5	15368.6	25601.5
4L Control	695.8	528.6	454.7	703.2	547.7	488.9	738.6	627.3	545.8
Wild-type	510.4	361.9	332.2	375.6	359.0	355.7	517.9	439.6	374.2

[0633] Taken together, these data support use of the 4L/PS-NA mice as model for neuropathic Gaucher disease, and for assessing efficacy of viral constructs encoding a GBA1 protein e.g., constructs GBA_VG1-GBA_VG34, e.g., as described in Tables 18-21 or 29-32 above.

Example 11: Exemplary Lead Identification

A. Generation of wild-type and enhanced GBA1 viral genome variants

[0634] Viral genomes were designed for AAV delivery of a GBA1 protein, e.g., a wild-type GBA1 protein (wtGBA) that does not further comprise an enhancement element; or an enhanced GBA1 protein (enGBA) that further comprises an enhancement element described herein, e.g., a prosaposin protein, a SapC protein, or functional variant thereof; a cell penetrating peptide (e.g., an ApoEII peptide, a TAT peptide, and/or an ApoB peptide) or functional variant thereof; a lysosomal targeting signal (LTS) or functional variant thereof; or a combination thereof (enGBAcombo). The nucleotide sequence from 5' ITR to 3' ITR of the viral genome constructs that comprise a transgene encoding an GBA1 protein with or without an enhancement element, are provided as GBA_VG1-GBA_VG33 herein, which are SEQ ID NOs: 1759-1771, 1809-1828, or 1870, respectively. These constructs are also summarized in Tables 5-6.

[0635] Each of these viral genome constructs comprise a nucleic acid comprising a transgene encoding a GBA1 protein. The transgene was designed to comprise a wild type nucleotide sequence encoding GBA1 (SEQ ID NO: 1777), or one of two different codon optimized nucleotide sequence encoding a GBA1 protein, SEQ ID NO: 1773 or 1781. In designing these viral genome constructs for expression of GBA, several promoters were selected and tested (e.g., promoters as described in Table 5), including a CMV promoter (SEQ ID NO: 1833); a CMVie enhancer and a CMV promoter (SEQ ID NO: 1831 and 1832, respectively); a CMVie enhancer and a CBA promoter (SEQ ID NO: 1831 and 1834 respectively); or an EF-1a promoter variant (SEQ ID NOs: 1839 or 1840).

[0636] Some of the viral genome constructs further comprised an intron region, of SEQ ID NO: 1842; a nucleotide sequence encoding a signal sequence (SEQ ID NO: 1850, 1851, or 1852); and/or 4 copies of a miR183 binding site (SEQ ID NO: 1847) separated by a spacer (SEQ ID NO: 1848), or miR183 binding series (SEQ ID NO: 1849). The viral constructs comprised a 5' ITR of SEQ ID NO: 1829; and a 3' ITR of SEQ ID NO: 1830. The polyadenylation sequence (SEQ ID NO: 1846) was the same across all viral genome constructs designed.

[0637] Wild-type GBA1 viral genome variants encoding a GBA1 protein were prepared as described and are outlined in Tables 5-6 (e.g., GBA_VG1, GBA_VG17-GBA_VG21, GBA_VG26, and GBA_VG33; SEQ ID NOs: 1759, 1812-1816, 1821, and 1828).

[0638] Enhanced GBA1 viral genome variants encoding a GBA1 protein and an enhancement element were prepared and are outlined in Table 5 (GBA_VG2-GBA_VG16, GBA_VG22-GBA_VG25, GBA_VG27-GBA_VG32; SEQ ID NO: 1760-1771, 1809-1811, 1817-1820, 1822-1827). The enhanced viral genomes were designed to further encode an enhancement element comprising a prosaposin protein (encoded by SEQ ID NO: 1859); saposin C protein or a functional variant (encoded by SEQ ID NO: 1787 or 1791); a cell penetrating peptide, including an ApoEII peptide (encoded by SEQ ID NO: 1797), a TAT protein (encoded by SEQ ID NO: 1793), or an ApoB peptide (encoded by SEQ ID NO: 1795); a lysosomal targeting signal (LTS) (encoded by any of SEQ ID NOs: 1799, 1801, 1803, 1805, or 1807); or a combination thereof. Some of the enhanced viral genome constructs further comprise a nucleotide sequence encoding a signal sequence (e.g., SEQ ID NO: 1856), and/or a linker (e.g., SEQ ID NO: 1724, 1726, or 1730). Some constructs, e.g., those encoding a prosaposin protein or a saposin C protein, encode a cleavable linker such as a furin and/or T2A cleavage site (encoded by SEQ ID NO: 1724 or 1726, respectively). Some constructs, e.g., those encoding a cell penetrating peptide, encode a flexible, glycine-serine linker (encoded by SEQ ID NO: 1730).

[0639] The viral construct GBA_VG1 (SEQ ID NO: 1759), comprising the nucleotide sequence of SEQ ID NO: 1781, with no additional enhancement elements (e.g., a saposin protein, a lysosomal targeting sequence, a cell penetrating sequence, or a combination thereof) was used as a reference or benchmark construct, e.g., in the experiments described herein.

B. In-vitro assessment of payload expression

[0640] Prior to vectorization, the wild-type and enhanced GBA1 viral genome variants were first used to validate the tools necessary for conducting lead identification studies, such as, but not limited to assays and cell-systems.

[0641] LC-MS/MS assays were established to quantify GBA1 (ng/mg of total protein) and SapC (ng/mg of total protein) in lysates collected from HEK293 cells transfected with a wild-type or enhanced GBA1 viral genome variant plasmid DNA including, GBA_VG1 (SEQ ID NO: 1759, encoding a GBA1 protein), GBA_VG8 (SEQ ID NO: 1766, encoding a GBA1 protein and a prosaposin protein of SEQ ID NO: 1785), GBA_VG9 (SEQ ID NO: 1767, encoding a GBA1 and a Saposin C protein of SEQ ID NO: 1789) and GBA_VG10 (SEQ ID NO: 1768, encoding a GBA1 protein and a Saponin C protein of SEQ ID NO: 1758). Lysates were also run on Western blot to confirm the presence of expressed GBA.

[0642] These validation experiments demonstrated that transfection of cells with the wild-type or enhanced GBA1 variant construct DNA resulted in increases in measured GBA1 or

SapC in the lysate as determined by LC-MS/MS and Western blot when compared to lysate of untransfected cells.

C. In-vitro cell-system assessment and validation

[0643] Additional LC-MS/MS assays were used to quantify GCase activity and/or levels of GBA1 substrates (*e.g.*, glycosphingolipids (GlcSph) quantified as ng/mg Actin in the FIG. 1A, or as ng/mg Lamp 1 in FIG. 1B) in Gaucher disease patient derived (GM04394-fibroblast (GD1 patient), GM00852-fibroblast (GD1 patient), GM00877-fibroblast (GD2 patient) or healthy control (GM05758-fibroblast from skin/inguinal area and GM02937-fibroblast from skin/unspecified) fibroblasts. Again, these quantifications were supplemented with Western blot analyses.

[0644] As anticipated, GBA1 substrate levels, specifically glycosphingolipids, as quantified as ng/mg Actin in FIG. 1A, or as ng/mg Lamp 1 in the in FIG. 1B, were increased across all three Gaucher disease patient derived fibroblast samples, as compared to control fibroblast levels, when measured by LC-MS/MS (FIGs. 1A-1B). Meanwhile, GBA1 protein detection (measured as the concentration of GBA1 in the cell lysate relative to total protein (ng/mg total protein) in GD patient fibroblasts was decreased compared to the healthy controls (FIG. 1C).

[0645] Quantification of GCase activity in lysate collected from Gaucher disease patient derived fibroblasts transfected with enhanced GBA1 viral genome variants was measured as Relative Fluorescence units per ng protein (RFU per ng protein) and was shown to be decreased when by 96.5%, 98.4% and 99.2% (GM04394-fibroblast, GM00852-fibroblast, GM00877-fibroblast, respectively) as compared to an average of the normal controls. Data are shown below in Table 16.

Table 16: Avg. GCase activity[RFU per ng protein] in Gaucher disease patient derived fibroblasts

Fibroblast cell line	GCase activity
GM04394	465.53
GM00852	221.29
GM00877	109.44
GM05758 (Control)	14997.59
GM02937 (Control)	11750.07

[0646] Quantification of GBA1 substrate in lysate collected from Gaucher disease patient derived fibroblasts transfected with enhanced GBA1 viral genome variants was measured as glucosylsphingosine/Lamp1 (ng/mg Lamp1) and was shown to be increased when compared to control. Data are shown below in Table 17.

Table 17: Avg. glucosylsphingosine (ng/mg Lamp1) in Gaucher disease patient derived

fibroblasts

Fibroblast cell line	Glucosylsphingosine
GM04394	716.33
GM00852	710.67
GM00877	4348.00
GM05758 (Control)	146.00
GM02937 (Control)	134.5

D. In-vitro packaging into AAV particles and capsid selection

[0647] The wild-type and enhanced GBA1 viral genome variants (GBA_VG1 to GBA_VG13; SEQ ID NO: 1759 - SEQ ID NO: 1771) were each packaged into AAV2 or AAV6 capsids.

[0648] *In vitro* capsid selection studies were conducted wherein cells were transduced with AAV particles comprising an enhanced GBA1 viral genome variant packaged in AAV2 or AAV6 at a series of increasing MOIs (1.00E1, 1.00E2, 1.00E3, 1.00E4, 1.00E5 and 1.00E6) and vector genome per cell quantified. Based on transduction efficiency, AAV2 was selected for further studies.

E. In-vitro dose-range finding studies

[0649] AAV particles comprising reference viral genome GBA_VG1 (SEQ ID NO: 1759) was screened in a dose-range finding study, wherein GCase activity was quantified subsequent to transduction of cells with an increasing series of MOIs (1.00E1, 1.00E2, 1.00E3, 1.00E4, 1.00E5 and 1.00E6). Based on these findings, a mid-range MOI of 1.00E3 was selected for further studies.

F. AAV2-GBA1 transduction in Gaucher Disease patient fibroblast cells

[0650] AAV2-GBA1 particles comprising viral genomes GBA_VG1 (SEQ ID NO: 1759), GBA_VG2 (SEQ ID NO: 1760), GBA_VG3 (SEQ ID NO: 1761), GBA_VG4 (SEQ ID NO: 1762), GBA_VG5 (SEQ ID NO: 1763), GBA_VG6 (SEQ ID NO: 1764), GBA_VG7 (SEQ ID NO: 1765) were administered at MOI 1.00E3 to Gaucher disease patient fibroblasts (GM00877-fibroblasts) and GCase activity quantified and normalized to mg protein. The results are shown in Table 18 below.

Table 18. GCase activity in Gaucher disease patient fibroblasts

Construct ID	SEQ ID NO:	GCase Activity [RFU per mL]
GBA_VG1	1759	9814.48
		16616.84
		3686.62
		3478.15
Avg.		8399.02

GBA_VG2	1760	6375.98
		3150.89
		7426.56
		-
Avg.		5651.14
GBA_VG3	1761	11580.6
		22812.97
		14152.31
		-
Avg.		16181.96
GBA_VG4	1762	10963.45
		11695.58
		7980.39
		-
Avg.		10213.14
GBA_VG5	1763	17076.48
		8665.12
		16343.61
		-
Avg.		14028.40
GBA_VG6	1764	5117.13
		3391.47
		4177.62
		-
Avg.		4228.74
GBA_VG7	1765	9842.00
		9366.20
		13187.36
		-
Avg.		10798.52

[0651] Western blot analysis further confirmed a ~70kD mature GBA1 protein in those samples transduced with AAV2-enhanced GBA1 particles. Negligible GBA1 was evident in Gaucher disease patient derived fibroblast samples that had not been transduced with an AAV2-enhanced GBA1 particle.

[0652] Additional AAV2 particles comprising viral genome constructs encoding a GBA1 protein and an enhancement element such as saposin C protein, a lysosomal targeting signal (LTS), a cell penetrating peptide (CPP), or a combination thereof (e.g., as outlined in Table 5 above), were vectorized for screening at an MOI of $10^{3.5}$ in Gaucher disease (GD) patient-derived fibroblasts (GD-II GM00877). The vectorized viral genome constructs included GBA_VG1 (SEQ ID NO: 1759), GBA_VG9 (SEQ ID NO: 1767), GBA_VG10 (SEQ ID NO: 1768), GBA_VG11 (SEQ ID NO: 1769), GBA_VG6 (SEQ ID NO: 1764), GBA_VG7 (SEQ ID

NO: 1765), GBA_VG12 (SEQ ID NO: 1770), GBA_VG3 (SEQ ID NO: 1761), GBA_VG4 (SEQ ID NO: 1762), GBA_VG5 (SEQ ID NO: 1763), and GBA_VG13 (SEQ ID NO: 1771). GCase activity was quantified in the pelleted patient cells (FIG. 2A) and the corresponding conditioned media (FIG. 2B), as relative fluorescence units per mg protein (RFU per mg protein). Treatment with six of the vectorized viral genome constructs (GBA_VG9, GBA_VG6, GBA_VG7, GBA_VG3, GBA_VG4, and GBA_VG5) resulted in an increase in GCase activity measured in the pelleted GD patient fibroblasts (FIG. 2A) and the corresponding conditioned media (FIG. 2B).

[0653] An LC-MS/MS assay was then used to quantify levels of the GBA1 substrate glucosylsphingosine (GlcSph, ng/mg Lamp1) in the cell lysis from GD II patient-derived fibroblasts transduced with the viral genomes constructs GBA_VG1 (SEQ ID NO: 1759), GBA_VG9 (SEQ ID NO: 1767), GBA_VG6 (SEQ ID NO: 1764), GBA_VG7 (SEQ ID NO: 1765), GBA_VG3 (SEQ ID NO: 1761), GBA_VG4 (SEQ ID NO: 1762), and GBA_VG5 (SEQ ID NO: 1763) vectorized in AAV2 particle. As shown in FIG. 3, the buildup of GBA1 substrate levels was reduced significantly in GD patient-derived fibroblasts transduced with the AAV2 GBA1 vectors, compared to the no AAV control. The data demonstrated that the AAV-mediated gene therapy can be effective in increasing GCase activity to treat diseases associated with GBA1 deficiency.

Example 12: AAV2 enhanced GBA1 vectors containing combinations of enhancement elements

[0654] Additional viral genome constructs were generated encoding a GBA1 protein, wherein the GBA1 protein is encoded by the wild-type nucleotide sequence of SEQ ID NO: 1777, or the codon optimized nucleotide sequence of SEQ ID NO: 1773, and further encoding an enhancement element such as a cell penetration peptide (CPP) (*e.g.*, ApoEII), a lysosomal targeting sequence (*e.g.*, LTS2), a SapC protein, or combination thereof. These constructs also comprised different promoters, including a CBA, a CMV, or a CAG promoter. These exemplary GBA1 viral genome constructs are included in Table 5-6.

[0655] GD-II patient fibroblasts (GM00877) were transduced with an AAV2 vector comprising the viral genome constructs: GBA_VG1 (SEQ ID NO: 1759), GBA_VG14 (SEQ ID NO: 1809), GBA_VG15 (SEQ ID NO: 1810), GBA_VG16 (SEQ ID NO: 1811), GBA_VG17 (SEQ ID NO: 1812), GBA_VG18 (SEQ ID NO: 1813), GBA_VG19 (SEQ ID NO: 1814), and GBA_VG20 (SEQ ID NO: 1815), at an MOI of $10^{2.5}$ (first bar), 10^3 (second bar), $10^{3.5}$ and 10^4 . The GCase activity was quantified on day 7 post-transduction after lysing the treated patient cells (FIG. 4A), as relative fluorescence units per mg protein (RFU per mg protein). As shown in

FIG. 4A, all viral genome constructs tested resulted in a dose-responsive increase in GCase activity in GD II patient-derived fibroblasts. The GBA_VG20 construct comprising the CAG promoter operably linked to a codon-optimized nucleotide sequence of SEQ ID NO: 1773 encoding a GBA1 protein vectorized in AAV2 vector showed significantly higher GCase activity compared to the GBA_VG1 construct comprising a CMVie enhancer and CBA promoter operably linked to the nucleotide sequence of SEQ ID NO: 1781 encoding the GBA1 protein at the MOI of 10^4 .

[0656] An LC-MS/MS assay was then used to quantify levels of the GBA1 substrate glucosylsphingosine (GlcSph, ng/mg Lamp1) in the cell lysis from GD II patient-derived fibroblasts transduced with the viral genomes constructs GBA_VG1 (SEQ ID NO: 1759), GBA_VG14 (SEQ ID NO: 1809), GBA_VG15 (SEQ ID NO: 1810), GBA_VG16 (SEQ ID NO: 1811), GBA_VG17 (SEQ ID NO: 1812), GBA_VG18 (SEQ ID NO: 1813), GBA_VG19 (SEQ ID NO: 1814), and GBA_VG20 (SEQ ID NO: 1815) vectorized in an AAV2 vector. As shown in FIG. 4B, all viral genome constructs tested reduced GBA1 substrate buildup indicating successful target engagement within GD patient cells.

Example 13: Bioinformatics Analysis of Wild-Type and Codon-Optimized Sequences Encoding a GBA1 Protein

[0657] Bioinformatics analysis on the sequence level was performed to differentiate between viral genome constructs encoding a GBA1 protein, wherein the GBA1 protein is encoded by a wild-type nucleotide sequence of SEQ ID NO: 1777 (e.g., the nucleotide sequence encoding the GBA1 protein of GBA_VG21, as shown in Table 5), a first codon-optimized nucleotide sequence of SEQ ID NO: 1773 (e.g., the nucleotide sequence encoding the GBA1 protein of GBA_VG17, as shown in Table 5-6), or a second codon-optimized of SEQ ID NO: 1781 (the nucleotide sequence encoding the GBA1 protein of GBA_VG1, as shown in Table 5). Briefly, sequence-level differentiation criteria, such as GC content, RNA accessibility, miRNA binding, transcriptional motifs and splicing events, were assessed using mRNA-based sequence analysis tools (RegRNA 2.0 by Chang *et al.*, 2013, *BMC Bioinformatics*, 14, Suppl 2:S4; miRDB by Chen & Wang, 2020, *Nucleic Acids Res*, 48(D1): D127–D131; the contents of each herein incorporated by reference in its entirety).

[0658] Based on the above analysis using miRDB (Chen & Wang, 2020, *supra*) with respect to miRNA binding, a series of putative recognition sites were found in the construct with first codon-optimized nucleotide sequence encoding a GBA1 protein of SEQ ID NO: 1773 (GBA_VG17). Specifically, this codon-optimized sequence of SEQ ID NO: 1773 had 42 total miRNA binding sites including 4 high confidence hits. Among those, 21 sites were distinct

from the second codon optimized sequence of SEQ ID NO: 1781 of GBA_VG1, and 11 sites were new and not present in the wild type nucleotide sequence of SEQ ID NO: 1777 of GBA_VG21. This is summarized in Table 19. A second miRNA analysis tool (RegRNA 2.0 by Chang et al., 2013 *supra*), as shown in Table 20, further confirmed that miRNA binding sites tended to be unique to each sequence codon optimized sequence analyzed.

Table 19. miRDB Summary of miRNA Binding

	WT (SEQ ID NO: 1777) vs SEQ ID NO: 1781	WT (SEQ ID NO: 1777) vs SEQ ID NO: 1773	SEQ ID NO: 1773 vs SEQ ID NO: 1781	“High Confidence” miRNA
SEQ ID NO: 1773 (GBA_VG17) Unique	N/A	30	21	4
Wild Type (SEQ ID NO: 1777) (GBA_VG21) Unique	42	41	N/A	1
SEQ ID NO: 1781 (GBA_VG1) Unique	27	N/A	17	2
Similar	11	12	21	0

Table 20. RegRNA 2.0 Summary of miRNA Binding

	WT (SEQ ID NO: 1777) vs SEQ ID NO: 1781	WT (SEQ ID NO: 1777) vs SEQ ID NO: 1773	SEQ ID NO: 1773 vs SEQ ID NO: 1781
SEQ ID NO: 1781 (GBA_VG1) Unique	3	N/A	3
SEQ ID NO: 1773 (GBA_VG17) Unique	N/A	2	3
WT (SEQ ID NO: 1777) (GBA_VG21) Unique	6	6	N/A
Similar	0	1	0

[0659] With respect to the analysis of the transcriptional motifs, the first codon-optimized sequence of SEQ ID NO: 1773 in GBA_VG17 had 70 total sites; 32 sites were distinct from the second codon optimized sequence of SEQ ID NO: 1781 in GBA_VG1, and 54 sites were new and not present in the wild type sequence of SEQ ID NO: 1777 in GBA_VG21 (Table 21).

Table 21. RegRNA 2.0 summary of regulatory motifs

	WT (SEQ ID NO: 1777) vs SEQ ID NO: 1781	WT (SEQ ID NO: 1777) vs SEQ ID NO: 1773	SEQ ID NO: 1773 vs SEQ ID NO: 1781
SEQ ID NO: 1781 (GBA_VG1) Unique	49	N/A	24
SEQ ID NO: 1773 (GBA_VG17) Unique	N/A	54	32
Wild Type (SEQ ID NO: 1777) (GBA_VG21) Unique	39	34	N/A
Similar	11	16	38

[0660] With respect to the splicing events analysis, the first codon-optimized sequence of SEQ ID NO: 1773 in GBA_VG17 had 5 total sites; 3 sites were distinct from the second codon-optimized sequence of SEQ ID NO: 1781 GBA1 sequence in GBA_VG1, and all 3 of these were completely new and not present in the wild-type sequence of SEQ ID NO: 1777 in GBA_VG21 (Table 22).

Table 22. Summary of RegRNA 2.0 Splice Events

	WT (SEQ ID NO: 1777) vs SEQ ID NO: 1781	WT (SEQ ID NO: 1777) vs SEQ ID NO: 1773	SEQ ID NO: 1773 vs SEQ ID NO: 1781
Wild Type (SEQ ID NO: 1777) (GBA_VG21) Unique	6	6	N/A
SEQ ID NO: 1773 (GBA_VG17) Unique	N/A	5	3
SEQ ID NO: 1781 (GBA_VG1) Unique	3	N/A	2
Similar	1	0	2

[0661] The GC content of the first codon optimized sequence (SEQ ID NO: 1773) was compared to the second codon optimized sequence (SEQ ID NO: 1781) and the wild-type sequence (SEQ ID NO: 1777). For RNA accessibility and GC content, the wild type GC biodistribution pattern was maintained in the first codon optimized sequence of SEQ ID NO: 1773 of GBA_VG17 (FIG. 5). However, the second codon optimized sequence of SEQ ID NO: 1781 (GBA_VG1) had balanced GC content across the entire length of the nucleotide sequence (FIG. 5).

[0662] The percentage homology for SEQ ID NO: 1781 (GBA_VG1) and SEQ ID NO: 1773 (GBA_VG17) with respect to the wild type sequence (SEQ ID NO: 1777; GBA_VG21) is

shown in Table 23. The first codon-optimized sequence of SEQ ID NO: 1773 in GBA_VG17 shares about 80.6% and about 80.0% sequence homology with respect to the wild type sequence of SEQ ID NO: 1777 in GBA_VG21, without and with the signal sequence, respectively. The second codon-optimized sequence (SEQ ID NO: 1781) in GBA_VG1 shares about 81.3% and about 80.7% sequence homology with respect to the wild type GBA1 sequence, without and with signal sequence, respectively. The first codon-optimized sequence of SEQ ID NO: 1773 in GBA_VG17 has about 87.0% and about 86.3% sequence homology with respect to the second codon optimized nucleotide sequence of SEQ ID NO: 1781, in GBA_VG1 without and with signal sequence, respectively. There were 131 unique mutations introduced into the first codon-optimized nucleotide sequence of SEQ ID NO: 1773 (GBA_VG17) relative to the wild type nucleotide sequence of SEQ ID NO: 1777 (GBA_VG21). The second codon-optimized nucleotide sequence of SEQ ID NO: 1781 (GBA_VG1) had 120 unique mutations relative to the wild type nucleotide sequence of SEQ ID NO: 1777 (GBA_VG21).

Table 23. GC Content and Percentage Homology of Codon Optimized Sequences (SEQ ID NO: 1773 or 1781) Relative to the Wild Type Sequence (SEQ ID NO: 1777)

% Homology to WT GBA					
cDNA Source	Without signal sequence	With Signal sequence	Splice site only	Unique Mutations	G/C content (WT 55%)
SEQ ID NO: 1773 (GBA_VG17)	80.6%	80.0%	4 bp mutation	131	59%
SEQ ID NO: 1781 (GBA_VG1)	81.3%	80.7%	5 bp mutation	120	58.5%
% SEQ ID NO: 1773 to SEQ ID NO: 1781 (GBA_VG17/GBA_VG1)	87.0%	86.3%	3 bp difference	190 (similar)	

Example 14: Functional Comparison of Wild-Type and Codon-Optimized Sequences Encoding a GBA1 Protein

[0663] GBA1 expression and GCase activity of a GBA1 protein was compared for the vectorized viral genome constructs GBA_VG17 (SEQ ID NO: 1812) comprising a first codon optimized sequence (SEQ ID NO: 1773) encoding the GBA1 protein, GBA_VG1 (SEQ ID NO: 1759) comprising a second codon optimized sequence (SEQ ID NO: 1781) encoding the GBA1

protein, and GBA_VG21 (SEQ ID NO: 1816) comprising a wild-type GBA1 sequence (SEQ ID NO: 1777) encoding a GBA1 protein.

[0664] GD-II patient fibroblasts (GD-II GM00877) were treated at $10^{4.5}$ MOI of AAV2 vectors comprising the following constructs: GBA_VG17 (AAV2.GBA_VG17), GBA_VG1 (AAV2.GBA_VG1), or GBA_VG21 (AAV2.GBA_VG21), and GCase activity was quantified as RFU per mL and normalized to mg of total protein. As shown in FIG. 6A, AAV2.GBA_VG17 resulted in superior enzymatic GCase activity compared to AAV2.GBA_VG1 and AAV2.GBA_VG21 treated cells. GCase activity was 52.4 fold higher in AAV2.GBA_VG17 treated GD patient cells compared to a no AAV control; but only 30.8 fold and 32.9 fold higher in AAV2.GBA_VG21 and AAV2.GB_VG1 treated GD patient cells, respectively, compared to a no AAV control.

[0665] GD-II patient fibroblasts (GD-II GM00877) were then treated at an MOI of 10^6 of AAV2 vectors comprising the following constructs: GBA_VG17 (AAV2.GBA_VG17), GBA_VG1 (AAV2.GBA_VG1), or GBA_VG21 (AAV2.GBA_VG21), and glucosylsphingosine levels (GlcSph in cell lysate (ng/mg Lamp1) and GBA1 substrate reduction activity was measured by LC-MS/MS. As shown in FIG. 6B, all constructs tested resulted in similar glucosylsphingosine levels and GBA1 substrate reduction, which were significantly reduced compared to the no AAV control. Expression of the encoded GBA1 protein by these GD patient cells treated with AAV2.GBA_VG17, AAV2.GBA_VG1, and AAV2.GBA_VG21 vectors was confirmed by Western blot.

[0666] Taken together, these data demonstrate higher GCase activity, stable GBA1 protein expression, and significant reduction of glucosylsphingosine levels with AAV2 vectorized GB_VG17 (SEQ ID NO: 1812), comprising the codon-optimized nucleotide sequence of SEQ ID NO: 1773 encoding the GBA1 protein compared to AAV2 vectorized GBA_VG1 and GBA_VG21.

Example 15. Route of Administration and Production Platform Comparison Study

[0667] In this Example, HEK and Sf9-produced AAV9 vectors, for biodistribution and GBA1 expression in wild type rat brain were assessed. The vectors were administered to the animals either by a single route of administration by intra-cisterna magna (ICM) or intra-thalamic (ITH) delivery, or a dual route of administration, comprising a combination of ICM and ITH delivery.

[0668] AAV9 vectors packaged with GBA_VG1 (SEQ ID NO: 1759) (AAV9.GBA_VG1) produced in HEK and SF9 cells were injected into wild-type rat. For bilateral ITH administration, 7.5×10^9 AAV9.GBA_VG1 viral genomes were injected into the thalamus of

each hemisphere, resulting in a total dose of 1.5×10^9 vector genomes. For ICM injection, 1.5×10^{10} AAV9.GBA_VG1 viral genomes were injected. For dual ITH and ICM administration, 1.5×10^{10} AAV9.GBA_VG1 viral genomes were injected for ICM delivery, and 7.5×10^9 AAV9.GBA_VG1 viral genomes were injected into the thalamus of each hemisphere for bilateral ITH delivery, for a total dose of 3×10^{10} AAV9.GBA_VG1 viral genomes. Four weeks post-injection, the brains of the rats were assayed for bio-distribution of the viral genome and GCase activity in the central nervous system and peripheral tissues.

[0669] First, all animals throughout all treatment groups continued to gain weight consistently post operatively until time of euthanasia (4 week in life). Daily clinical observations showed normal healthy subjects. Therefore, at both selected doses of 1.5×10^{10} for single route of administration, and 3×10^{10} for combination route of administration, all animals tolerated the AAV treatments.

[0670] Viral genome distribution was also assessed at 28 days post intrathalamic dosing of HEK and Sf9 produced AAV9-GBA1 vectors in the wild-type rat brain, particularly in the thalamus, hippocampus striatum, and cortex. In the thalamus, hippocampus and striatum, there was a trend of higher average biodistribution with HEK-produced AAV9 vector compared to Sf9 (~3-6 fold VG/cell increase). In the cortex, a similar average biodistribution profile was observed for HEK and Sf9 produced AAV9 vectors. These data show increased biodistribution with AAV9-GBA1 vectors produced by an HEK platform as compared to Sf9 following intrathalamic dosing in rats.

[0671] GCase activity was also compared at 28 days post intrathalamic dosing of HEK and Sf9 produced AAV9-GBA1 vectors in the wild-type rat brain. GCase activity was generally increased over baseline. However, the average increase in GCase activity was the greatest in the thalamus (site of injection) (70% over endogenous for Sf9 produced vectors and 20% over endogenous for HEK vectors, and in the thalamus, a moderate increase in average GCase activity was observed for HEK produced vectors relative to Sf9 produced vectors. Low to moderate increase was observed in forebrain and midbrain regions (cortex, striatum and hippocampus, ~5-40% over endogenous).

[0672] Taken together, these data demonstrate that bilateral ITH AAV9.GBA_VG1 dosing resulted in successful distribution of AAV VGs in the CNS tissues and detectable overexpression of GBA1 (increased GCase activity over endogenous GCase activity) within a wild-type rat brain.

[0673] The effects of routes of administration on viral genome distribution and GCase activity of HEK produced AAV9.GBA_VG1 vectors were evaluated at day 28 post ICM, ITH,

or dual ICM and ICM delivery of the vectors. A significantly higher viral genome distribution in ITH group was observed in deep-brain structures, including the thalamus and hippocampus, compared to ICM or dual ITH and ICM dosing. Similarly, higher viral genome distribution was observed in ITH group compared to ICM and dual ITH and ICM dosing. For cortex tissue, dual ITH and ICM dosing resulted in a significantly higher viral genome biodistribution compared to ITH and ICM dosing. Taken together, ITH delivery of AAV9-GBA1 vector displayed a higher viral genome (VG) biodistribution profile in the deep-midbrain structures (especially in the hippocampus and thalamus) as compared to ICM and dual ITH + ICM delivery. Further, ITH delivery appeared to drive the VG biodistribution profile in fore/mid brain observed with dual ITH + ICM injection.

[0674] For hindbrain tissues, a significantly higher cerebellar viral genome distribution was observed in ITH group compared to ICM or dual ITH and ICM dosing. Similarly, a trend of higher viral genome biodistribution was observed in brainstem for ITH group compared to ICM and dual ITH and ICM dosing. Therefore, similar to forebrain and midbrain structures, ITH delivery of AAV9-GBA_VG1 vector produced by HEK cells displayed a higher viral genome biodistribution profile in the hindbrain structure as compared to ICM and dual ITH and ICM delivery.

[0675] For forebrain and midbrain tissues with respect to GCase activity, the highest increase was observed at site of injection in thalamus (about 250% post ITH deliver followed by about 207% in dual ITH and ICM dosing). Moderate increase was observed post ITH delivery in cortex (about 123% compared to vehicle control, and about 141% after dual ITH and ICM dosing). For ICM dosing, minimal or no increase in GCase activity was observed in thalamus (about 110%) and cortex (about 91%) post ICM delivery. Combinatorial dosing (ICM and ITH) showed the highest GCase activity in cortex (about 141%). Overall, based on the viral genome distribution results and the GCase results, ITH dosing appears to be driving the increase in GCase activity in thalamus and cortex. Additionally, the AAV genome per cell biodistribution shows similar trend of GCase activity in the thalamus and cortex.

[0676] For hindbrain tissues, the highest increase was observed post ITH injection in cerebellum (about 178%), and a low increase was seen post both ICM and dual ICM and ITH delivery in cerebellum. Combinatorial dosing group did not show a higher increase in GCase activity readout within cerebellum. Overall, based on the viral genome distribution results and the GCase results, ITH dosing appears to be driving the increase in GCase activity in the cerebellum and the AAV genome per cell biodistribution shows similar trend of GCase activity in the cerebellum.

[0677] GCase activity was also evaluated in CSF fluid. Different levels of increase in CSF GCase activity were observed with different routes of administration. Specifically, the highest increase in GCase activity was observed in combinatorial dosing (ITH and ICM), followed by ITH delivery, and only moderate increase was observed by ICM delivery. These data demonstrated that AAV delivered GBA1 gene transfer in rats resulted in secretion of active GCase product in CSF.

[0678] This experiment demonstrated that intrathalamic injection and dual mode injection resulted in a more efficient delivery of AAV-GBA1 viral particles and a higher GCase expression/activity in the CNS tissues and the CSF.

Example 16: *In vivo* Evaluation of a Vectorized Viral Genome Comprising a Codon Optimized Nucleotide Sequence Encoding GBA1

[0679] This Example investigates the distribution and efficacy of the viral genome construct GB_VG17 (SEQ ID NO: 1812) comprising a codon-optimized nucleotide sequence (SEQ ID NO: 1773) encoding a GBA1 protein, vectorized in a VOY101 capsid (VOY101.GBA_VG17) in wild-type C57BL/6 mice. VOY101 is a capsid protein that enables blood brain barrier penetration after IV injection.

[0680] Mice were intravenously injected with 2×10^{13} VG/kg of VOY101.GBA_VG17 or a vehicle control, into the lateral tail vein. At 28-days post IV injection, various CNS tissues (e.g., cortex, striatum, hippocampus, thalamus, cerebellum, brainstem, and/or spinal cord) and peripheral tissues (e.g., heart, liver, and/or spleen) were harvested to measure viral genome (VG) biodistribution (VG/cell), GCase activity, and GBA1 mRNA expression (transgene specific and endogenous). All animals treated remained healthy and there was no significant difference in the body weight between mice treated with VOY101.GBA_VG17 and mice treated with the vehicle control.

[0681] With respect to VG biodistribution, high levels (approximately >50 vg/cell) of VOY101.GBA_VG17 distribution was observed across the forebrain and midbrain. In the cortex, 81.31 VG/cell were quantified on average (range: ~ 75 -85 vg/cell); in the striatum, 150.39 VG/cell were quantified on average (range: ~ 90 -330 vg/cell); in the hippocampus, 152.91 VG/cell were quantified on average (range: ~ 70 -195 vg/cell); and in the thalamus, 117.94 VG/cell were quantified on average (range: ~ 70 -190 vg/cell). Therefore, successful VOY101.GBA_VG17 gene transfer was achieved across the forebrain and midbrain regions.

[0682] Similarly, high levels (approximately >50 vg/cell) of VOY101.GBA_VG17 distribution was observed across the hind brain and spinal cord (cervical region). In the cerebellum, 65.77 VG/cell were quantified on average (range: ~ 23 -105 vg/cell); in the

brainstem, 159.22 VG/ cell were quantified on average (range: ~ 110-305 vg/cell); and in the spinal cord, 176.29 VG/cell were quantified on average (range: ~95-280 vg/cell). Therefore, successful VOY101.GBA_VG17 gene transfer was also achieved across the hindbrain and spinal cord.

[0683] With respect to VG biodistribution in peripheral tissues, detectable levels of VOY101.GBA_VG17 were observed in the heart, spleen, and liver, but this was approximately 4-10 fold lower than the levels observed in the CNS tissues. In the heart, 11.58 VG/cell were quantified on average (range: ~5-21 vg/cell); in the spleen, 29.99 VG/cell were quantified on average (range: ~ 7-82 vg/cell); and in the liver, 18.76 VG/ cell were quantified on average (range: ~ 5-60 vg/cell).

[0684] With respect to GCase activity (measured as RFU/mL normalized to mg of protein), the forebrain and midbrain following IV injection of VOY101.GBA_VG17 demonstrated a significant increase in GCase activity over baseline (vehicle control). The highest increase was observed in the cortex (4.86 fold higher than the vehicle control). A similar increase was observed in the striatum (4.6 fold higher than the vehicle control) and the thalamus (4.74 fold higher than the vehicle control). Therefore, a significant increase in GCase activity was observed in the forebrain and midbrain which had high VOY101.GBA_VG17 biodistribution. Similarly, the hindbrain structures following IV injection of VOY101.GBA_VG17 demonstrated a significant increase in GCase activity over baseline (vehicle control). The cerebellum showed a 4.04 fold higher GCase activity than the vehicle control and the brainstem showed a 5.26 fold higher GCase activity than the vehicle control. Therefore, a significant increase in GCase activity was also observed in the hind brain which had high VOY101.GBA_VG17 biodistribution. Overall, all brain regions tested showed a 4-5 fold increase in GCase activity relative to the vehicle control and IV delivery of VOY101.GBA_VG17 resulted in a successful and uniform increase in GCase activity across pertinent CNS tissues.

[0685] GCase activity was also measured in the liver (as RFU/mL normalized to mg of protein) following IV injection of VOY101.GBA_VG17. The liver showed a 4.12 fold increase in GCase activity relative to the vehicle control, demonstrating a successful increase in GBA1 activity in a non-CNS tissue.

[0686] In addition to the cellular and tissue GCase activity levels quantified in both the CNS and the liver, GCase activity was also measured in the fluid of the mice, including in the cerebral spinal fluid (CSF) and the serum, post-IV injection of VOY101.GBA_VG17. GCase activity was higher in the serum as compared to the CSF. A 5.9 fold increase in GCase activity relative to the vehicle treated control was observed in the CSF and a 22.3 fold increase in GCase

activity relative to the vehicle treated control was observed in the serum. These data demonstrate active GCase is secreted into extracellular compartments following IV injection of VOY101.GBA_VG17.

[0687] The GCase activity levels quantified and the fold increase in activity relative to the vehicle in the CNS and peripheral tissues and fluid measured following IV injection of VOY101.GBA_VG17 are summarized in Table 24.

Table 24. Summary of GCase activity levels (RFU/per mL) normalized to mg of protein

GCase Activity (RFU/per mL) normalized to mg of protein		Fold Increase in GCase activity relative to no vehicle control
Cortex	Vehicle	4.86
62246	12789	
Striatum	Vehicle	4.64
32063	6898	
Thalamus	Vehicle	4.74
40506	9061	
Cerebellum	Vehicle	4.04
36082	8926	
Brainstem	Vehicle	5.26
44706	8495	
Liver	Vehicle	4.1
100123	24272	
CSF	Vehicle	5.9
3707	627	
Serum	Vehicle	22.3
6919	310	

[0688] Both endogenous and transgene specific GBA1 mRNA (payload) expression was quantified post-IV injection of VOY101.GBA_VG17 in the cortex, thalamus, and brainstem. GBA1 mRNA was quantified as GBA1 mRNA expression per 1,000 transcripts normalized to geomean (GAPDH, HPRT1, PPIA). With respect to endogenous GBA1 mRNA, approximately 38-63 copies per 1000 transcripts were measured across the brain. More specifically, in the cortex, thalamus, and brainstem, 63.10 endogenous GBA1 mRNA/1,000 transcripts, 38.81 endogenous GBA1 mRNA/1,000 transcripts, and 38.95 endogenous GBA1 mRNA/1,000 transcripts were quantified, respectively. With respect transgene specific GBA1 mRNA, approximately 1314 – 1765 copies per 1000 transcripts were measured across the brain. In the cortex, thalamus, and brainstem, 1314.39 transgene specific GBA1 mRNA/1,000

transcripts, 1547.21 transgene specific GBA1 mRNA/1,000 transcripts, and 1764.02 transgene specific GBA1 mRNA/1,000 transcripts were quantified, respectively. Accordingly, in the cortex, thalamus, and brain stem, there was an 874 fold, 1032 fold, and 1244 fold increase in transcript specific GBA1 mRNA compared to the vehicle control, respectively. Therefore, successful transcription of the transgene comprising the codon-optimized nucleotide sequence (SEQ ID NO: 1773) encoding the GBA1 protein was achieved in the brain at 28-days post IV injection of a blood brain barrier penetrant VOY101.GBA_VG17 vector. Endogenous GBA1 mRNA levels in the brain maintained similar levels in the tissues treated with VOY101.GBA_VG17 and the tissues treated with the vehicle control. These data demonstrate successful transgene transcription and expression of a GBA1 payload in the CNS following IV injection of VOY101.GBA_VG17.

[0689] Both endogenous and transgene specific GBA1 mRNA expression was also quantified post-IV injection of VOY101.GBA_VG17 in the liver. In the liver, 182.29 endogenous GBA1 mRNA/1,000 transcripts were quantified (range: ~188 – 240 per 1000 transcripts), and there was no significant difference in the endogenous GBA1 mRNA levels between treated and untreated mice. Approximately, 1372.45 transgene specific GBA1 mRNA/1,000 transcripts were quantified in the liver, and a 739 fold increase in GBA1 mRNA was observed in the treated mice compared to the vehicle control. These data demonstrate successful transgene transcription and expression of a GBA1 payload in the liver following IV injection of VOY101.GBA_VG17.

[0690] The relationship between biodistribution (VG/cell) and GCase activity (RFU/mL, fold over endogenous GCase activity, normalized to mg of protein) following IV injection of VOY101.GBA_VG17 was also evaluated in the cortex, striatum, thalamus, brainstem, cerebellum, and liver of the mice. In the CNS tissues, approximately a 300-660% fold increase in GCase activity over endogenous GCase activity was observed (FIG. 8) and 595-1825 transgene-specific GBA1 mRNA copies/1000 transcripts were quantified. In the liver, while there was intra-group variability, a 180-850% fold increase in GCase activity relative to endogenous GCase activity was measured, with approximately 330-2450 transgene specific GBA1 mRNA copies per 1000 transcripts quantified. The GCase levels quantified in the liver were comparable to the CNS tissues at lower VG/cell levels (FIG. 8). It is predicted that a 30-50% fold increase in GCase activity over endogenous is clinically impactful. Therefore, intravenous injection of VOY101.GBA_VG17 was able to increase the levels of GCase activity in various CNS and peripheral tissues relative to endogenous, well above the predicted fold increase thought to be clinically impactful.

[0691] Some of these data discussed above are summarized in Table 25 below. In summary,

VOY101.GBA_VG17 which comprises the codon-optimized nucleotide sequence of SEQ ID NO: 1773 encoding the GBA1 protein demonstrated high biodistribution in the CNS, increased GCase activity in the CNS and peripheral tissues and fluid, and successful transgene transcription and expression. GBA_VG17 could therefore be used in the treatment of disorders associated with a lack of a GBA1 protein and/or GCase activity, such as neuronopathic (affects the CNS) and non-neuronopathic (affects non-CNS) Gaucher's disease, PD associated with a mutation in the GBA1 gene, and dementia with Lewy Bodies.

Table 25: Summary of VG biodistribution, GCase activity, and GBA1 mRNA data 28 day post IV injection of VOY101.GBA_VG17 at 2e13 vg/kg in the cortex, thalamus, brain stem and liver

Region	VG/Cell	GBA1 mRNA (over endogenous GBA)	GBA1 mRNA (over vehicle baseline)	GCase expression (over vehicle baseline)
Cortex	83.31	20.83	874	4.86
Thalamus	117.94	39.87	1032	4.74
Brain Stem	159.22	45.29	1244	5.26
Liver	18.76	7.29	739	4.12

Example 17. De-targeting GBA1 Expression in the Dorsal Root Ganglia (DRG)

[0692] This Example demonstrates the use of a miR183 binding site to reduce GBA1 expression in the dorsal root ganglion (DRG) neurons, which express the corresponding endogenous microRNA, miR183.

[0693] A viral genome construct, GBA_VG33 (SEQ ID NO: 1828, described in Tables 18-19 and 21), comprising a codon-optimized nucleotide sequence (SEQ ID NO: 1773) encoding a GBA1 protein and a miR183 binding site series (SEQ ID NO: 1849), comprising four miR183 binding sites (each comprising SEQ ID NO: 1847), each separated by an 8 nucleotide spacer (SEQ ID NO: 1848). The GBA_VG33 were also vectorized into an AAV2 vector (AAV2.GBA_VG33).

[0694] HEK293 cells were transfected with the GBA_VG33 construct and GBA1 protein expression was compared to cells transfected with the GB_VG17 control construct (SEQ ID NO: 1812) which comprises a codon-optimized nucleotide sequence (SEQ ID NO: 1773) encoding a GBA1 protein but does not comprise a miR183 binding site series. Similar GBA1 protein expression levels were observed following transfection with the GBA_VG33 construct and the GBA_VG17 control construct. HEK293 cells were also co-transfected with either the GBA_VG33 construct comprising the miR183 binding site series, or the GBA_VG17 control construct, and miR183, and GBA1 protein expression was measured. A significant reduction of GBA1 expression was observed in HEK293 cells co-transfected the GBA_VG33 construct and miR183 compared to those cells co-transfected with the GBA_VG17 control and miR183. These

data demonstrated that the GBA_VG33 construct comprising the miR183 binding site series was able to reduce GBA1 expression in the presence of the corresponding microRNA (miR183).

[0695] De-targeting of GBA1 expression in the DRG was also investigated in rat embryonic DRG neurons. The rat embryonic DRG neurons were transduced with AAV2.GBA_VG33 (comprises the miR183 binding site series) or AAV2.GBA_VG17 control at an MOI of $10^{3.5}$ or $10^{4.5}$, or a no AAV control. GCase activity was measured as RFU/mL per mg of total protein. As shown in FIG. 7, GCase activity was significantly reduced in the rat embryonic DRG neurons transduced with AAV2.GBA_VG33 compared to those transfected with AAV2.GBA_VG17, at both MOIs tested. Also, the levels of GCase activity measured in the rat embryonic DRG neurons transduced with AAV2.GBA_VG33 at both MOIs was similar to the level of GCase measured in the no AAV control.

[0696] Taken together, these data demonstrate that successful GBA1 expression de-targeting in the DRG was achieved with the GBA_VG33 (SEQ ID NO: 1828), comprising a codon-optimized nucleotide sequence (SEQ ID NO: 1773) encoding the GBA1 protein and the miR183 binding site series (SEQ ID NO: 1849).

Example 18: *In vivo* Evaluation of a Vectorized Viral Genome Comprising a Codon-optimized, CpG Depleted Nucleotide Sequence Encoding GBA1 in Wildtype Mice

[0697] This Example investigates the distribution and efficacy of two viral genome constructs: GB_VG35 (SEQ ID NO: 2006) and GB_VG36 (SEQ ID NO: 2007), each comprising a codon-optimized, CpG-depleted nucleotide sequence (SEQ ID NO: 2002) encoding a GBA1 protein and vectorized in a VOY101 capsid (VOY101.GBA_VG35 and VOY101.GBA_VG36) in wild-type C57BL/6 mice. In addition, the viral construct VOY101.GBA_VG17 is also assessed.

[0698] Mice were intravenously injected with 2×10^{13} VG/kg of VOY101.GBA_VG35, VOY101.GBA_VG36, VOY101.GBA_VG17 (see Example 16), or a vehicle control, into the lateral tail vein. At 28-days post IV injection, various CNS tissues (e.g., cortex, striatum, hippocampus, thalamus, cerebellum, brainstem, and/or spinal cord) and peripheral tissues (e.g., heart, liver, and/or spleen) were harvested to measure viral genome (VG) biodistribution (VG/cell), GCase activity, and GBA1 mRNA expression (transgene specific and endogenous).

[0699] VG biodistributions in the cortex, brainstem, and striatum are shown in FIG. 9. All VG values are presented as mean and standard deviation and statistical analysis was done using 1-way ANOVA and Tukey HSD post-hoc test.

[0700] GCase activities (measured as nM 4mu pdct/mg protein) in the cortex, striatum, and brainstem are shown in FIG. 10.

Example 19: *In vivo* Evaluation of a Vectorized Viral Genome Comprising a Codon-optimized, CpG Depleted Nucleotide Sequence Encoding GBA1 in 4L-PS/NA Mice

[0701] This Example investigates the distribution, GCase activities, and substrate reduction of two viral genome constructs: GB_VG35 (SEQ ID NO: 2006) and GB_VG36 (SEQ ID NO: 2007), each comprising a codon-optimized, CpG-depleted nucleotide sequence (SEQ ID NO: 2002) encoding a GBA1 protein and vectorized in a single-stranded VOY101 capsid (ssVOY101.GBA_VG35 and ssVOY101.GBA_VG36) in 4L-PS/NA mice. In addition, single-stranded VOY101 comprising a codon-optimized nucleotide sequence (SEQ ID NO: 1773) encoding a GBA1 protein is also assessed (ssVOY101.GBA_VG17).

[0702] Mice were intravenously administered 2×10^{13} VG/kg of one of the three viral genome constructs or a vehicle control via lateral tail injection. At 28-days post IV injection, various CNS tissues (e.g., cortex, striatum, hippocampus, thalamus, cerebellum, brainstem, and/or spinal cord) and peripheral tissues (e.g., heart, liver, and/or spleen) were harvested to measure viral genome (VG) biodistribution (VG/cell), GCase activity, and substrate reduction.

[0703] Biodistributions and GCase activities in the brainstem and DRGs are shown in FIG. 11. ssVOY101.GBA_VG36 showed significant reduction in GBA1 expression & GCase activities in DRGs, but retain expression and activities in brain stem.

[0704] The substrates glucosylceramide and glucosylsphingosine were quantified in the brain stem, striatum, and DRGs by LC/MS-MS. Significant substrate reduction was observed in both brain stem and striatum with all three constructs (FIG. 12). DRG substrates were not significantly changed. Also, the significantly reduced GBA1 mRNA levels obtained in DRGs using ssVOY101.GBA_VG36 resulted in reduced potential toxicity. These data demonstrate that both ssVOY101.GBA_VG35 and ssVOY101.GBA_VG36 exhibited a desired biodistribution profile and were effective in reducing substrate levels in brain tissues.

Example 20: *In vivo* Evaluation of a Vectorized Viral Genome Comprising an HA-tagged Nucleotide Sequence Encoding GBA1 in Wildtype Mice

[0705] This Example investigates the distribution and efficacy of GBA_VG17-VP and GBA_VG17-VE comprising a codon-optimized nucleotide sequence (SEQ ID NO: 1773) encoding a GBA1 protein, and GBA_VG17-HA comprising a codon-optimized nucleotide sequence (SEQ ID NO: 1773) encoding a GBA1 and further comprising an HA-tag. Both constructs were vectorized in a VOY101 capsid (VOY101.GBA_VG17 and VOY101.GBA_VG17-HA) for administration in wild-type C57BL/6 mice.

[0706] Mice were intravenously injected with 2×10^{13} VG/kg of VOY101.GBA_VG17 and VOY101.GBA_VG17-HA into the lateral tail vein. At 28-days post IV injection, various CNS

tissues (e.g., cortex, striatum, hippocampus, thalamus, cerebellum, brainstem, and/or spinal cord) and peripheral tissues (e.g., heart, liver, and/or spleen) were harvested to measure viral genome (VG) biodistribution (VG/cell), GCase activity, and GBA1 mRNA expression (transgene specific and endogenous).

[0707] Biodistribution in the cortex and GCase activities in the cortex, striatum, and brainstem are shown in FIG. 13. VOY101.GBA_VG17-HA showed comparable GCase activity as VOY101.GB-VG17.

[0708] Immunohistochemical assessments of GBA1 or GBA1-HA expression in the cortex, striatum, brainstem, cerebellum, thalamus, and hippocampus are shown in FIGs. 14A and 14B. VOY101.GBA_VG17-HA showed robust HA expression in all regions evaluated. No HA signal was detected in mice injected with VOY101.GBA_VG17.

IX. Equivalents and Scope

[0709] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with the Detailed Description provided herein. The scope of the present disclosure is not intended to be limited to the above Detailed Description, but rather is as set forth in the appended claims.

[0710] In the claims, articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The disclosure includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The disclosure includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process.

[0711] It is also noted that the term “comprising” is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term “comprising” is used herein, the term “consisting of” is thus also encompassed and disclosed.

[0712] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[0713] In addition, it is to be understood that any particular embodiment of the present

disclosure that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the disclosure (*e.g.*, any, composition, therapeutic or active ingredient; any method of production; any method of use; *etc.*) can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[0714] It is to be understood that the words which have been used are words of description rather than limitation, and that changes may be made within the purview of the appended claims without departing from the true scope and spirit of the disclosure in its broader aspects.

[0715] While the present disclosure has been described at some length and with some particularity with respect to the several described embodiments, it is not intended that it should be limited to any such particulars or embodiments or any particular embodiment, but it is to be construed with references to the appended claims so as to provide the broadest possible interpretation of such claims in view of the prior art and, therefore, to effectively encompass the intended scope of the disclosure.

[0716] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, section headings, the materials, methods, and examples are illustrative only and not intended to be limiting.

CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising a nucleotide sequence that encodes a β -glucocerebrosidase 1 (GBA1) protein and is at least 93% identical (e.g., at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence of SEQ ID NO: 2002.
2. The isolated nucleic acid of claim 1, wherein the nucleotide sequence encoding the GBA1 protein comprises a nucleotide sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to SEQ ID NO: 2002.
3. The isolated nucleic acid of claim 1 or claim 2, wherein the nucleotide sequence encoding the GBA1 protein comprises SEQ ID NO: 2002.
4. The isolated nucleic acid of any one of claims 1-3, further comprising a signal sequence comprising a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to SEQ ID: 2005.
5. The isolated nucleic acid of claim 4, wherein the signal sequence comprises the nucleotide sequence of SEQ ID NO: 2005.
6. An isolated nucleic acid comprising a nucleotide sequence that encodes a β -glucocerebrosidase 1 (GBA1) protein and is at least 94% identical (e.g., at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence of SEQ ID NO: 2001.
7. The isolated nucleic acid of claim 6, wherein the nucleotide sequence encoding the GBA1 protein comprises the nucleotide sequence of SEQ ID NO: 2001.
8. A recombinant viral genome comprising a nucleotide sequence encoding a β -glucocerebrosidase 1 (GBA1) protein, wherein the recombinant viral genome further comprises an miRNA (miR) binding site that modulates expression of the encoded GBA1 protein in a cell or tissue of the DRG, liver, hematopoietic lineage, or a combination thereof; wherein the

recombinant viral genome comprises a GBA1-encoding nucleotide sequence that is at least 93% identical (e.g., at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence of SEQ ID NO: 2002.

9. The recombinant viral genome of claim 8, wherein the nucleotide sequence encoding the GBA1 protein comprises a nucleotide sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence of SEQ ID NO: 2002.

10. The recombinant viral genome of claim 8 or claim 9, wherein the nucleotide sequence encoding the GBA1 protein comprises or consists of the nucleotide sequence of SEQ ID NO: 2002.

11. The recombinant viral genome of any one of claims 8-10, further comprising a promoter operably linked to the nucleic acid comprising the nucleotide sequence encoding the GBA protein, wherein, optionally, the promoter comprises a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence of SEQ ID NO: 1834, wherein, further optionally, the promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 1834.

12. The recombinant viral genome of any one of claims 8-11, further comprising an enhancer.

13. The recombinant viral genome of claim 12, wherein the enhancer comprises a CMVie enhancer; wherein, optionally, the CMVie enhancer comprises a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence of SEQ ID NO: 1831; wherein, further optionally, the CMVie enhancer comprises or consists of the nucleotide sequence of SEQ ID NO: 1831.

14. The recombinant viral genome of any one of claims 8-13, further comprising an intron sequence; wherein, optionally, the intron sequence comprises a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the

nucleotide sequence of SEQ ID NO: 1842; wherein, further optionally, the intron sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 1842.

15. The recombinant viral genome of any one of claims 8-14, further comprising a polyadenylation (polyA) sequence; wherein, optionally, the polyA sequence comprises a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence of SEQ ID NO: 1846; wherein, further optionally, the polyA sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 1846.

16. The recombinant viral genome of any one of claims 8-15, further comprising an ITR sequence; wherein, optionally, the ITR sequence comprises a nucleotide sequence that is at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence of SEQ ID NO: 1829 or SEQ ID NO: 1830; wherein, further optionally, the ITR sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 1829 or SEQ ID NO: 1830.

17. The recombinant viral genome of claim 16, comprising a 5' ITR sequence and a 3' ITR sequence, wherein the 5' ITR sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 1829 and the 3' ITR comprises or consists of the nucleotide sequence of SEQ ID NO: 1830.

18. The recombinant viral genome of any one of claims 8-17, further comprising one or more miR183 binding sites; wherein, optionally, the viral genome comprises four miR183 binding sites; wherein, further optionally, each of the four miR183 binding sites comprises or consists of the nucleotide sequence of SEQ ID NO: 1847 or a nucleotide sequence that has up to 3 modifications relative to SEQ ID NO: 1847.

19. The recombinant viral genome of any one of claims 8-17, further comprising a miR183 binding site series comprising a nucleotide sequence that is at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence of SEQ ID NO: 1849; wherein, optionally, the miR183 binding site series comprises or consists of the nucleotide sequence of SEQ ID NO: 1849.

20. The recombinant viral genome of any one of claims 8-17, wherein the viral genome does not comprise a miR183 binding site.

21. A recombinant viral genome comprising, in 5' to 3' order:

(i) a 5' adeno-associated (AAV) ITR comprising or consisting of the nucleotide sequence of SEQ ID NO: 1829 or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(ii) a CMV_{ie} enhancer comprising or consisting of the nucleotide sequence SEQ ID NO: 1831 or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iii) a CB promoter comprising or consisting of the nucleotide sequence of SEQ ID NO: 1834, or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iv) an intron comprising or consisting of the nucleotide sequence of SEQ ID NO: 1842, or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(v) a nucleotide sequence encoding a signal sequence, wherein the nucleotide sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 2005, or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(vi) a nucleotide sequence encoding a GBA1 protein, wherein the nucleotide sequence encoding the GBA1 protein comprises or consists of the nucleotide sequence of SEQ ID NO: 2002 or a nucleotide sequence at least 93% identical (e.g., at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(vii) a polyA signal region comprising or consisting of the nucleotide sequence of SEQ ID NO: 1846, or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and

(viii) a 3' AAV ITR comprising or consisting of the nucleotide sequence of SEQ ID NO: 1830, or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

22. The recombinant viral genome of claim 21, wherein:

(i) the 5' AAV ITR comprises or consists of the nucleotide sequence of SEQ ID NO: 1829;

- (ii) the CMVie enhancer comprises or consists of the nucleotide sequence of SEQ ID NO: 1831;
- (iii) the CB promoter or functional variant thereof comprises or consists of the nucleotide sequence of SEQ ID NO: 1834;
- (iv) the intron comprises or consists of the nucleotide sequence of SEQ ID NO: 1842;
- (v) the nucleotide sequence encoding the signal sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 2005;
- (vi) the nucleotide sequence encoding the GBA1 protein comprises or consists of the nucleotide sequence of SEQ ID NO: 2002;
- (vii) the polyA signal region comprises or consists of the nucleotide sequence of SEQ ID NO: 1846; and
- (viii) the 3' AAV ITR comprises or consists of the nucleotide sequence of SEQ ID NO: 1830.

23. The recombinant viral genome of claim 22, comprising the nucleotide sequence of SEQ ID NO: 2006, or a nucleotide sequence at least 97% identical (e.g., at least 97%, at least 98%, or at least 99% identical) thereto.

24. The recombinant viral genome of claim 22 or claim 23, comprising the nucleotide sequence of SEQ ID NO: 2006.

25. The recombinant viral genome of any one of claims 22-24, consisting of the nucleotide sequence of SEQ ID NO: 2006.

26. A recombinant viral genome comprising in 5' to 3' order:

- (i) a 5' adeno-associated (AAV) ITR comprising or consisting of the nucleotide sequence of SEQ ID NO: 1829, or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;
- (ii) a CMVie enhancer comprising or consisting of the nucleotide sequence of SEQ ID NO: 1831, or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;
- (iii) a CB promoter comprising or consisting of the nucleotide sequence of SEQ ID NO: 1834, or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(iv) an intron comprising or consisting of the nucleotide sequence of SEQ ID NO: 1842, or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(v) a nucleotide sequence encoding a signal sequence, optionally wherein the nucleotide sequence encoding the signal sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 2005, or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(vi) a nucleotide sequence encoding a GBA1 protein comprising or consisting of the nucleotide sequence of SEQ ID NO: 2002 or a nucleotide sequence at least 93% identical (e.g., at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the nucleotide sequence of SEQ ID NO: 2002;

(vii) a miR183 binding site series comprising or consisting of the nucleotide sequence of SEQ ID NO: 1849 or a nucleotide sequence at least 90% identical (e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto;

(viii) a polyA signal region comprising or consisting of the nucleotide sequence of SEQ ID NO: 1846, or a nucleotide sequence at least 95% identical (e.g., at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto; and

(ix) a 3' AAV ITR comprising or consisting of the nucleotide sequence of SEQ ID NO: 1830, or a nucleotide sequence at least 95% identical (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) thereto.

27. The recombinant viral genome of claim 26, wherein:

(i) the 5' AAV ITR comprises or consists of the nucleotide sequence of SEQ ID NO: 1829;

(ii) the CMVie enhancer comprises or consists of the nucleotide sequence of SEQ ID NO: 1831;

(iii) the CB promoter or functional variant thereof comprises or consists of the nucleotide sequence of SEQ ID NO: 1834;

(iv) the intron comprises or consists of the nucleotide sequence of SEQ ID NO: 1842;

(v) the nucleotide sequence encoding the signal sequence comprises the nucleotide sequence of SEQ ID NO: 2005;

(vi) the nucleotide sequence encoding the GBA1 protein comprises or consists of the nucleotide sequence of SEQ ID NO: 2002;

(vii) the miR183 binding site series comprises or consists of the nucleotide sequence of SEQ ID NO: 1849;

(viii) the polyA signal region comprises or consists of the nucleotide sequence of SEQ ID NO: 1846; and

(ix) the 3' AAV ITR comprises or consists of the nucleotide sequence of SEQ ID NO: 1830.

28. The recombinant viral genome of claim 27, comprising the nucleotide sequence of SEQ ID NO: 2007, or a nucleotide sequence at least 97% identical (e.g., at least 97%, at least 98%, or at least 99% identical) thereto.

29. The recombinant viral genome of claim 27 or claim 28, comprising the nucleotide sequence of SEQ ID NO: 2007.

30. The recombinant viral genome of claim 27 or claim 28, consisting of the nucleotide sequence of SEQ ID NO: 2007.

31. The recombinant viral genome of any one of claims 8-30, further comprising a nucleic acid encoding a capsid protein, wherein the capsid protein comprises a VP1 polypeptide, a VP2 polypeptide, and/or a VP3 polypeptide.

32. The recombinant viral genome of claim 31, wherein the VP1 polypeptide, the VP2 polypeptide, and/or the VP3 polypeptide are encoded by at least one Cap gene.

33. The recombinant viral genome of any one of claims 8-32, further comprising a nucleic acid encoding a Rep protein, wherein the Rep protein comprises a Rep78 protein, a Rep68, Rep52 protein, and/or a Rep40 protein.

34. The recombinant viral genome of claim 33, wherein the Rep78 protein, the Rep68 protein, the Rep52 protein, and/or the Rep40 protein are encoded by at least one Rep gene.

35. An AAV particle comprising:

(i) a capsid protein; and

(ii) the recombinant viral genome of any one of claims 8-30.

36. The AAV particle of claim 35, wherein the capsid protein comprises a VOY101, VOY201, AAVPHP.N (PHP.N), AAVPHP.B (PHP.B), AAVPHP.A (PHP.A), PHP.B2, PHP.B3, G2B4, G2B5, AAV5, AAV9, AAVrh10 capsid protein, or a functional variant thereof.
37. The AAV particle of claim 35 or claim 36, wherein the capsid protein comprises a variant of an AAV5 capsid protein.
38. The AAV particle of claim 35 or claim 36, wherein the capsid protein comprises a variant of an AAV9 capsid protein.
39. A vector comprising the isolated nucleic acid of any one of claims 1-7 or the recombinant viral genome of any one of embodiments 8-34.
40. A cell comprising the isolated nucleic acid of any one of claims 1-7, the recombinant viral genome of any one of claims 8-34, the viral particle of any one of claims 35-38, or the vector of claim 39, wherein, optionally, the cell is a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell.
41. A nucleic acid comprising the recombinant viral genome of any one of embodiments 8-34, and a backbone region suitable for replication of the viral genome in a cell, e.g., a bacterial cell (e.g., wherein the backbone region comprises one or both of a bacterial origin of replication and a selectable marker).
42. A method of making a recombinant AAV particle, the method comprising
- (i) providing a host cell comprising the recombinant viral genome of any one of claims 8-34; and
 - (ii) incubating the host cell under conditions suitable to enclose the viral genome in a capsid protein;
- thereby making the isolated AAV particle;
- wherein, optionally, the capsid protein is a VOY101 capsid protein.
43. The method of claim 42, further comprising, prior to step (i), introducing a first nucleic acid molecule comprising the viral genome into the host cell.

44. The method of claim 43, wherein the host cell comprises a second nucleic acid molecule encoding a capsid protein, wherein, optionally, the capsid protein is a VOY101 capsid protein.
45. The method of claim 43, further comprising introducing the second nucleic acid into the cell.
46. The method of claim 44 or claim 45, wherein the second nucleic acid molecule is introduced into the host cell prior to, concurrently with, or after the first nucleic acid molecule.
47. The method of any one of claims 42-46, wherein the host cell comprises a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell.
48. A pharmaceutical composition comprising the AAV particle of any one of claims 35-38, and a pharmaceutically acceptable excipient.
49. A method of delivering a nucleic acid sequence encoding a GBA1 protein to a subject, comprising administering an effective amount of the pharmaceutical composition of claim 48, the AAV particle of any one of claims 35-38, the isolated nucleic acid of any one claims 1-7, or the recombinant viral genome of any one of claims 8-34, thereby delivering the nucleic acid encoding a GBA1 protein to the subject.
50. The method of claim 49, wherein the subject has, has been diagnosed with having, or is at risk of having a disease associated with expression of GBA1, e.g., aberrant or reduced GBA1 expression, e.g., expression of an GBA1 gene, GBA1 mRNA, and/or GBA1 protein.
51. The method of claim 49 or claim 50, wherein the subject has, has been diagnosed with having, or is at risk of having a neurodegenerative or neuromuscular disorder.
52. A method of treating a subject having or diagnosed with having a disease associated with GBA1 expression comprising administering an effective amount of the pharmaceutical composition of claim 48, the AAV particle of any one of claims 35-38, the isolated nucleic acid of any one claims 1-7, or the recombinant viral genome of any one of claims 8-34, thereby treating the disease associated with GBA1 expression in the subject.

53. A method of treating a subject having or diagnosed with having a neurodegenerative or neuromuscular disorder, comprising administering an effective amount of the pharmaceutical composition of claim 48, the AAV particle of any one of claims 35-38, the isolated nucleic acid of any one claims 1-7, or the recombinant viral genome of any one of claims 8-34, thereby treating the neurodegenerative or neuromuscular disorder in the subject.

54. The method of any one of claims 49-53, wherein the disease associated with GBA1 expression or the neurodegenerative or neuromuscular disorder is Parkinson's Disease (PD).

55. The method of any one of claims 49-53, wherein the disease associated with GBA1 expression or the neurodegenerative or neuromuscular disorder is Gaucher Disease (GD).

56. The method of claim 55, wherein the GD is type 1 GD (GD1) or type 3 GD (GD3).

57. The method of any one of claims 49-53, wherein the disease associated with GBA1 expression or the neurodegenerative or neuromuscular disorder is Dementia with Lewy Bodies (DLB).

58. A method of treating a subject having or diagnosed with having Parkinson's Disease (PD) comprising administering an effective amount of the pharmaceutical composition of claim 48, the AAV particle of any one of claims 35-38, the isolated nucleic acid of any one claims 1-7, or the recombinant viral genome of any one of claims 8-34, thereby treating PD in the subject.

59. A method of treating a subject having or diagnosed with having Gaucher Disease (GD) comprising administering an effective amount of the pharmaceutical composition of claim 48, the AAV particle of any one of claims 35-38, the isolated nucleic acid of any one claims 1-7, or the recombinant viral genome of any one of claims 8-34, thereby treating GD in the subject.

60. The method of claim 59, wherein the GD is GD1 or GD3.

61. A method of treating a subject having or diagnosed with having Dementia with Lewy Bodies (DLB) comprising administering an effective amount of the pharmaceutical composition of claim 48, the AAV particle of any one of claims 35-38, the isolated nucleic acid of any one

claims 1-7, or the recombinant viral genome of any one of claims 8-34, thereby treating DLB in the subject.

62. The method of any one of claims 49-61, wherein the subject has a reduced level of GCase activity as compared to a reference level; wherein, optionally, the level of GCase activity is measured by a 4-MUG assay or a SensoLyte Blue Glucocerebrosidase assay.

63. The method of claim 62, wherein the reference level comprises the level of GCase activity in a healthy subject who does not have a disease associated with GBA1 expression, a neuromuscular disorder, and/or a neurodegenerative disorder.

64. The method of any one of claims 49-63, wherein treating comprises prevention of progression of the disease in the subject.

65. The method of any one of claims 49-64, wherein treating results in amelioration of at least one symptom of the disease associated with GBA1 expression, the neurodegenerative disorder, and/or the neuromuscular disorder in the subject.

66. The method of claim 65, wherein the symptom of the disease associated with GBA1 expression, the neurodegenerative disorder, and/or the neuromuscular disorder comprises reduced GCase activity, accumulation of glucocerebroside and other glycolipids, e.g., within immune cells (e.g., macrophages), build-up of synuclein aggregates (e.g., Lewy bodies), developmental delay, progressive encephalopathy, progressive dementia, ataxia, myoclonus, oculomotor dysfunction, bulbar palsy, generalized weakness, trembling of a limb, depression, visual hallucinations, cognitive decline, or a combination thereof.

67. The method of any one of claims 49-66, wherein the subject is a human subject.

68. The method of any one of claims 49-67, wherein the subject has one or more mutations in a GBA1 gene, a GBA1 mRNA, and/or a GBA1 protein.

69. The method of any one of claims 49-68, wherein the pharmaceutical composition, the AAV particle, the isolated nucleic acid, or the recombinant viral genome is administered to the subject intravenously, intracerebrally, via intrathalamic (ITH) administration, intramuscularly,

intrathecally, intracerebroventricularly, via intraparenchymal administration, via focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration, or via intra-cisterna magna injection (ICM).

70. The method of any one of claims 49-69, wherein the pharmaceutical composition, the AAV particle, the isolated nucleic acid, or the recombinant viral genome is administered to the subject intravenously.

71. The method of any one of claims 49-70, wherein the pharmaceutical composition, the AAV particle, the isolated nucleic acid, or the recombinant viral genome is delivered to a cell, tissue, or region of the CNS, e.g., a region of the brain or spinal cord, e.g., the parenchyma, the cortex, substantia nigra, caudate cerebellum, striatum, corpus callosum, cerebellum, brain stem caudate-putamen, thalamus, superior colliculus, the spinal cord, or a combination thereof.

72. The method of any one of claims 49-71, which further comprises evaluating, e.g., measuring, the level of GBA1 expression, e.g., GBA1 gene, GBA1 mRNA, and/or GBA1 protein expression, in the subject, e.g., in a cell, tissue, or fluid, of the subject, optionally wherein the level of GBA1 protein is measured by an assay described herein, e.g., an ELISA, a Western blot, or an immunohistochemistry assay.

73. The method of claim 72, wherein measuring the level of GBA1 expression is performed prior to, during, or subsequent to treatment with the AAV particle.

74. The method of 72 or 73, wherein the cell or tissue is a cell or tissue of the central nervous system (e.g., parenchyma).

75. The method of any one of claims 49-74, wherein the administration results in increased level of GBA1 protein expression in a cell or tissue of the subject, relative to reference level, e.g., a subject that has not received treatment, e.g., has not been administered the AAV particle.

76. The method of any one of claims 49-75, which further comprises evaluating, e.g., measuring, the level of GCase activity in the subject, e.g., in a cell or tissue of the subject, optionally

wherein the level of GCase activity is measured by a 4-MUG assay or a SensoLyte Blue Glucocerebrosidase assay.

77. The method of any one of claims 49-76, wherein the administration results in an increase in at least one, two, or all of:

(i) the level of GCase activity in a cell, tissue, (e.g., a cell or tissue of the CNS, e.g., the cortex, striatum, thalamus, cerebellum, and/or brainstem), and/or fluid (e.g., CSF and/or serum), of the subject, optionally wherein the level of GCase activity is increased by at least 2, 3, 4, or 5 fold, as compared to a reference level, e.g., a subject that has not received treatment, e.g., has not been administered the AAV particle;

(ii) the level of viral genomes (VG) per cell in a CNS tissue (e.g., the cortex, striatum, thalamus, cerebellum, brainstem, and/or spinal cord) of the subject, optionally wherein the VG level is increased by greater than 50 VGs per cell, as compared to a peripheral tissue, wherein the level of VGs per cell is at least 4-10 fold lower than the levels in the CNS tissue, e.g., as measured by an assay as described herein; and/or

(iii) the level of GBA1 mRNA expression in a cell or tissue (e.g., a cell or tissue of the CNS, e.g., the cortex, thalamus, and/or brainstem), optionally wherein the level of GBA1 mRNA is increased by at least 100-1300 fold as compared to a reference level from a subject that has not received the treatment.

78. The method of any one of claims 49-77, further comprising administration of an additional therapeutic agent and/or therapy suitable for treatment or prevention of the disease associated GBA1 expression, the neurodegenerative disorder, and/or the neuromuscular disorder; wherein, optionally, the additional therapeutic agent and/or therapy comprises enzyme replacement therapy (ERT) (e.g., imiglucerase, velaglucerase alfa, or taliglucerase alfa); substrate reduction therapy (SRT) (e.g., eliglustat or miglustat), blood transfusion, levodopa, carbidopa, Safinamide, dopamine agonists (e.g., pramipexole, rotigotine, or ropinirole), anticholinergics (e.g., benztropine or trihexyphenidyl), cholinesterase inhibitors (e.g., rivastigmine, donepezil, or galantamine), an N-methyl-d-aspartate (NMDA) receptor antagonist (e.g., memantine), or a combination thereof.

79. The pharmaceutical composition of claim 48, the AAV particle of any one of claims 35-38, the isolated nucleic acid of any one claims 1-7, or the recombinant viral genome of any one of claims 8-34, for use in the manufacture of a medicament.

80. The pharmaceutical composition of claim 48, the AAV particle of any one of claims 35-38, the isolated nucleic acid of any one claims 1-7, or the recombinant viral genome of any one of claims 8-34, for use in the treatment of a disease associated with GBA1 expression, a neuromuscular and/or a neurodegenerative disorder; wherein, the disease associated with GBA1 expression, a neuromuscular and/or a neurodegenerative disorder is PD.

81. Use of an effective amount of the pharmaceutical composition of claim 48, the AAV particle of any one of claims 35-38, the isolated nucleic acid of any one claims 1-7, or the recombinant viral genome of any one of claims 8-34, for use in the manufacture of a medicament for the treatment of a disease associated with GBA1 expression, a neuromuscular and/or a neurodegenerative disorder; wherein, the disease associated with GBA1 expression, a neuromuscular and/or a neurodegenerative disorder is PD.

82. The pharmaceutical composition of claim 48, the AAV particle of any one of claims 35-38, the isolated nucleic acid of any one claims 1-7, or the recombinant viral genome of any one of claims 8-34, for use in the treatment of a disease associated with GBA1 expression, a neuromuscular and/or a neurodegenerative disorder; wherein, the disease associated with GBA1 expression, a neuromuscular and/or a neurodegenerative disorder is GD.

83. The pharmaceutical composition, the AAV particle, the isolated nucleic acid, or the recombinant viral genome for use of claim 82, wherein the GD is GD1.

84. The pharmaceutical composition, the AAV particle, the isolated nucleic acid, or the recombinant viral genome for use of claim 82, wherein the GD is GD3.

85. Use of an effective amount of the pharmaceutical composition of claim 48, the AAV particle of any one of claims 35-38, the isolated nucleic acid of any one claims 1-7, or the recombinant viral genome of any one of claims 8-34, for use in the manufacture of a medicament for the treatment of a disease associated with GBA1 expression, a neuromuscular and/or a neurodegenerative disorder; wherein, the disease associated with GBA1 expression, a neuromuscular and/or a neurodegenerative disorder is GD.

86. The use of claim 85, wherein the GD is GD1.

87. The use of claim 85, wherein the GD is GD3.