CLAIMS

1. An isolated nucleic acid comprising a nucleotide sequence that encodes a β-glucocerebrosidase 1 (GBA1) protein and is 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%, 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%, 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: 2005.

5. The isolated nucleic acid of claim 4, wherein the signal sequence comprises the nucleotide sequence of SEQ ID NO: 2005.

6. The isolated nucleic acid of claim 5, 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 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

7. A recombinant viral genome comprising the isolated nucleic acid of any one of claims 1-6.

8. The recombinant viral genome of claim 7, further comprising a nucleotide sequence encoding an miRNA (miR) binding site that modulates expression of the encoded GBA1 protein in a cell or tissue of the dorsal root ganglia (DRG), liver, hematopoietic lineage, or a combination thereof.

9. The recombinant viral genome of claim 7 or claim 8, further comprising a promoter operably linked to the nucleotide sequence encoding the GBA1 protein, wherein the promoter comprises a nucleotide 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%, at least 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 1834.

10. The recombinant viral genome of claim 9, wherein the promoter comprises the nucleotide sequence of SEQ ID NO: 1834.

11. The recombinant viral genome of any one of claims 7-10, further comprising an enhancer, wherein the enhancer comprises a nucleotide 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%, at least 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 1831.

12. The recombinant viral genome of claim 11, wherein the enhancer comprises the nucleotide sequence of SEQ ID NO: 1831.

13. The recombinant viral genome of any one of claims 7-12, further comprising an intron sequence, wherein the intron sequence comprises a nucleotide 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%, at least 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 1842.

14. The recombinant viral genome of claim 13, wherein the intron comprises the nucleotide sequence of SEQ ID NO: 1842.

15. The recombinant viral genome of any one of claims 7-14, further comprising a polyadenylation (polyA) sequence wherein the polyA sequence comprises a nucleotide 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%, at least 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 1846.

16. The recombinant viral genome of claim 15, wherein the polyA sequence comprises the nucleotide sequence of SEQ ID NO: 1846.

17. The recombinant viral genome of any one of claims 7-16, further comprising an ITR sequence, wherein the ITR sequence comprises a nucleotide 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%, at least 99%, or 100% identical to the nucleotide sequence of SEQ ID NO: 1829 or SEQ ID NO: 1830.

18. The recombinant viral genome of claim 17, wherein the ITR sequence comprises the nucleotide sequence of SEQ ID NO: 1829 or SEQ ID NO: 1830.

19. The recombinant viral genome of any one of claims 7-18, comprising a 5' ITR sequence and a 3' ITR sequence, wherein the 5' ITR sequence comprises the nucleotide sequence of SEQ ID NO: 1829 and the 3' ITR comprises the nucleotide sequence of SEQ ID NO: 1830.

20. The recombinant viral genome of any one of claims 7-19, further comprising one or more nucleotide sequences encoding one or more miR183 binding sites.

21. The recombinant viral genome of claim 20, wherein the viral genome comprises four nucleotide sequences, wherein each of the four nucleotide sequences encodes one of four miR183 binding sites, and wherein each of the four nucleotide sequences comprises the nucleotide sequence of SEQ ID NO: 1847 or a nucleotide sequence that has up to 3 modifications relative to SEQ ID NO: 1847.

22. The recombinant viral genome of any one of claims 7-21, comprising a nucleotide sequence encoding a miR183 binding site series comprising a nucleotide sequence that is 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.

23. The recombinant viral genome of claim 7 or any one of claims 9-19, wherein the viral genome does not comprise a nucleotide sequence encoding a miR183 binding site.

24. The recombinant viral genome of any one of claims 7-23, comprising, in 5’ to 3’ order:

(i) a 5’ ITR comprising the nucleotide sequence of SEQ ID NO: 1829 or a nucleotide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto;

(ii) an enhancer comprising the nucleotide sequence of SEQ ID NO: 1831 or a nucleotide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto;

(iii) a promoter comprising the nucleotide sequence of SEQ ID NO: 1834, or a nucleotide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto;

(iv) an intron comprising the nucleotide sequence of SEQ ID NO: 1842, or a nucleotide sequence 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 the nucleotide sequence of SEQ ID NO: 2005, or a nucleotide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto;

(vi) the nucleotide sequence encoding a GBA1 protein;

(vii) a polyA signal region comprising the nucleotide sequence of SEQ ID NO: 1846, or a nucleotide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto; and

(viii) a 3’ ITR comprising the nucleotide sequence of SEQ ID NO: 1830, or a nucleotide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

25. The recombinant viral genome of claim 24, wherein:

(i) the 5’ ITR comprises the nucleotide sequence of SEQ ID NO: 1829;

(ii) the enhancer comprises the nucleotide sequence of SEQ ID NO: 1831;

(iii) the 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’ ITR comprises the nucleotide sequence of SEQ ID NO: 1830.

26. The recombinant viral genome of any one of claims 7-25, comprising the nucleotide sequence of SEQ ID NO: 2006, or a nucleotide sequence at least 97%, at least 98%, or at least 99% identical thereto.

27. The recombinant viral genome of any one of claims 7-26, comprising the nucleotide sequence of SEQ ID NO: 2006.

28. The recombinant viral genome of any one of claims 7-27, consisting of the nucleotide sequence of SEQ ID NO: 2006.

29. The recombinant viral genome of any one of claims 7-22, 24, and 25, comprising in 5’ to 3’ order:

(i) a 5’ ITR comprising the nucleotide sequence of SEQ ID NO: 1829, or a nucleotide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto;

(ii) an enhancer comprising the nucleotide sequence of SEQ ID NO: 1831, or a nucleotide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto;

(iii) a promoter comprising the nucleotide sequence of SEQ ID NO: 1834, or a nucleotide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto;

(iv) an intron comprising the nucleotide sequence of SEQ ID NO: 1842, or a nucleotide sequence 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 comprising the nucleotide sequence of SEQ ID NO: 2005, or a nucleotide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto;

(vi) the nucleotide sequence encoding a GBA1 protein;

(vii) a nucleotide sequence encoding a miR183 binding site series comprising the nucleotide sequence of SEQ ID NO: 1849 or a nucleotide sequence 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 the nucleotide sequence of SEQ ID NO: 1846, or a nucleotide sequence 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’ ITR comprising the nucleotide sequence of SEQ ID NO: 1830, or a nucleotide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

30. The recombinant viral genome of claim 29, wherein:

(i) the 5’ ITR comprises the nucleotide sequence of SEQ ID NO: 1829;

(ii) the enhancer comprises the nucleotide sequence of SEQ ID NO: 1831;

(iii) the 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 nucleotide sequence encoding 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’ ITR comprises the nucleotide sequence of SEQ ID NO: 1830.

31. The recombinant viral genome of any one of claims 7-22, 24, 25, 29, and 30, comprising the nucleotide sequence of SEQ ID NO: 2007, or a nucleotide sequence at least 97%, at least 98%, or at least 99% identical thereto.

32. The recombinant viral genome of any one of claims 7-22, 24, 25, and 29-31, comprising the nucleotide sequence of SEQ ID NO: 2007.

33. The recombinant viral genome of any one of claims 7-22, 24, 25, and 29-32, consisting of the nucleotide sequence of SEQ ID NO: 2007.

34. An adeno-associated virus (AAV) particle comprising:

(i) an AAV capsid protein; and

(ii) a recombinant viral genome comprising the isolated nucleic acid of any one of claims 1-6 or the recombinant viral genome of any one of claims 7-33.

35. The AAV particle of claim 34, wherein the AAV capsid protein comprises an AAV9 capsid protein or a variant of an AAV9 capsid protein.

36. A vector comprising the isolated nucleic acid of any one of claims 1-6 or the recombinant viral genome of any one of claims 7-33.

37. A cell comprising the isolated nucleic acid of any one of claims 1-6, the recombinant viral genome of any one of claims 7-33, the viral particle of claim 34 or claim 35, or the vector of claim 36, wherein the cell is a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell.

38. A method of making a recombinant adeno-associated virus (AAV) particle, the method comprising

(i) providing a host cell comprising a recombinant viral genome comprising the isolated nucleic acid of any one of claims 1-6 or comprising the recombinant viral genome of any one of claims 7-33; and

(ii) incubating the host cell under conditions that encapsulate the viral genome in an AAV capsid protein;

thereby making the recombinant AAV particle.

39. The method of claim 38, further comprising, prior to step (i), introducing a first nucleic acid molecule comprising the recombinant viral genome into the host cell.

40. The method of claim 39, wherein the host cell comprises a second nucleic acid molecule encoding the AAV capsid protein.

41. The method of claim 40, further comprising introducing the second nucleic acid into the cell.

42. The method of claim 40 or claim 41, wherein the second nucleic acid molecule is introduced into the host cell concurrently with the first nucleic acid molecule.

43. The method of any one of claims 38-42, 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.

44. A pharmaceutical composition comprising the AAV particle of claim 34 or claim 35, and a pharmaceutically acceptable excipient.

45. Use of the isolated nucleic acid of any one of claims 1-6, the recombinant viral genome of any one of claims 7-33, the AAV particle of claim 34 or claim 35, the vector of claim 36, the cell of claim 37, or the pharmaceutical composition of claim 44 in the manufacture of a medicament for delivering a nucleotide sequence encoding a GBA1 protein to a subject.

46. The use of claim 45, wherein the subject has, has been diagnosed with having, or is at risk of having a disease associated with GBA1 expression, e.g., aberrant or reduced GBA1 expression, e.g., expression of a GBA1 gene, GBA1 mRNA, and/or GBA1 protein.

47. The use of claim 45 or claim 46, wherein the subject has, has been diagnosed with having, or is at risk of having a neurodegenerative or neuromuscular disorder.

48. Use of the isolated nucleic acid of any one of claims 1-6, the recombinant viral genome of any one of claims 7-33, the AAV particle of claim 34 or claim 35, the vector of claim 36, the cell of claim 37, or the pharmaceutical composition of claim 44 in the manufacture of a medicament for treating a subject having or diagnosed with having a disease associated with GBA1 expression, e.g., expression of a GBA1 gene, GBA1 mRNA, and/or GBA1 protein.

49. Use of the isolated nucleic acid of any one of claims 1-6, the recombinant viral genome of any one of claims 7-33, the AAV particle of claim 34 or claim 35, the vector of claim 36, the cell

of claim 37, or the pharmaceutical composition of claim 44 in the manufacture of a medicament for treating a subject having or diagnosed with having a neurodegenerative or neuromuscular disorder.

50. The use of any one of claims 46-49, wherein the disease associated with GBA1 expression or the neurodegenerative or neuromuscular disorder is Parkinson's Disease (PD).

51. The use of any one of claims 46-49, wherein the disease associated with GBA1 expression or the neurodegenerative or neuromuscular disorder is Gaucher Disease (GD).

52. The use of claim 51, wherein the GD is type 1 GD (GD1), type 2 GD (GD2), or type 3 GD (GD3).

53. The use of any one of claims 46-49, wherein the disease associated with GBA1 expression or the neurodegenerative or neuromuscular disorder is Dementia with Lewy Bodies (DLB).

54. Use of the isolated nucleic acid of any one of claims 1-6, the recombinant viral genome of any one of claims 7-33, the AAV particle of claim 34 or claim 35, the vector of claim 36, the cell of claim 37, or the pharmaceutical composition of claim 44 in the manufacture of a medicament for treating a subject having or diagnosed with having Parkinson's Disease (PD).

55. Use of the isolated nucleic acid of any one of claims 1-6, the recombinant viral genome of any one of claims 7-33, the AAV particle of claim 34 or claim 35, the vector of claim 36, the cell of claim 37, or the pharmaceutical composition of claim 44 in the manufacture of a medicament for treating a subject having or diagnosed with having Gaucher Disease (GD).

56. The use of claim 55, wherein the GD is type 1 GD (GD1), type 2 GD (GD2), or type 3 GD (GD3).

57. Use of the isolated nucleic acid of any one of claims 1-6, the recombinant viral genome of any one of claims 7-33, the AAV particle of claim 34 or claim 35, the vector of claim 36, the cell of claim 37, or the pharmaceutical composition of claim 44 in the manufacture of a medicament for treating a subject having or diagnosed with having Dementia with Lewy Bodies (DLB).

58. The use of any one of claims 45-57, wherein the subject has a reduced level of β-glucocerebrosidase (GCase) activity as compared to a reference level.

59. The use of claim 58, 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.

60. The use of any one of claims 48-57, wherein the treating comprises prevention of progression of the disease or disorder in the subject.

61. The use of any one of claims 48-57, wherein the 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.

62. The use of claim 61, 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.

63. The use of any one of claims 45-62, wherein the subject is a human subject.

64. The use of any one of claims 45-63, wherein the subject has one or more mutations in a GBA1 gene, a GBA1 mRNA, and/or a GBA1 protein.

65. The use of any one of claims 45-64, wherein the medicament is suitable for administration 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).

66. The use of any one of claims 45-65, wherein the medicament is suitable for administration to the subject intravenously.

67. The use of any one of claims 45-66, wherein the use comprises delivery 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.

68. The use of any one of claims 48-67, wherein the treating 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.

69. The use of claim 68, wherein measuring the level of GBA1 expression is performed prior to, during, or subsequent to treatment with the AAV particle.

70. The use of claim 68 or claim 69, wherein the cell or tissue is a cell or tissue of the central nervous system (e.g., parenchyma).

71. The use of any one of claims 48-70, wherein the treating results in increased level of GBA1 protein expression in a cell or tissue of the subject, relative to a reference level, e.g., a subject that has not received treatment, e.g., has not been administered the AAV particle.

72. The use of any one of claims 46-71, wherein the use 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.

73. The use of any one of claims 48-72, wherein the treating 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.

74. The use of any one of claims 48-73, wherein the treating further comprises administration of an additional therapeutic agent and/or therapy suitable for treatment or prevention of the disease associated with 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.