CLAIMS

1. An adeno-associated virus (AAV) particle comprising an AAV capsid variant and a viral genome, wherein the viral genome comprises a frataxin (FXN)-encoding sequence and the AAV capsid variant comprises an amino acid sequence having the formula [N2]-[N3], wherein:

(i) [N2] comprises X_1, X_2, X_3, X_4, and X_5, wherein:

(a) X_1 is Y, N, or C;

(b) X_2 is P, K, T, or Q;

(c) X_3 is A or P;

(d) X_4 is E, S, or A; and

(e) X_5 is V, L, or E; and

(ii) [N3] comprises the amino acid sequence VQK, EQK, VKK, VHK, VQQ, or LQK; and

wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 739, or an amino acid sequence at least 95% identical thereto.

2. The AAV particle of claim 1, wherein [N2]-[N3] is present in loop VIII, wherein loop VIII is present at amino acids comprising those corresponding to positions 571-599 of the amino acid sequence of SEQ ID NO: 982.

3. The AAV particle of claim 1 or claim 2, wherein [N2]-[N3] is present immediately subsequent to an amino acid corresponding to position 576 of the amino acid sequence of SEQ ID NO: 982.

4. The AAV particle of any one of claims 1-3, wherein the AAV capsid variant is an AAV5 capsid variant comprising [N2]-[N3] in place of an amino acid corresponding to T577 of the amino acid sequence of SEQ ID NO: 138.

5. The AAV particle of any one of claims 1-4, wherein [N2] comprises the amino acid sequence YP, YPA, YPAE (SEQ ID NO: 21), or YPAEV (SEQ ID NO: 1).

6. The AAV particle of any one of claims 1-5, wherein [N3] comprises the amino acid sequence VQK.

7. The AAV particle of any one of claims 1-6, wherein [N2]-[N3] comprises the amino acid sequence AEVVQK (SEQ ID NO: 36) or PAEVVQK (SEQ ID NO: 20).

8. The AAV particle of any one of claims 1-7, wherein [N2]-[N3] comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943).

9. The AAV particle of any one of claims 1-8, wherein the AAV capsid variant further comprises [N1] comprising X_D, X_E, and X_F, wherein:

(a) X_D of [N1] is Q, T, S, A, I, L, or H;

(b) X_E of [N1] is S, G, A, or R; and

(c) X_F of [N1] is S, K, L, R, A, or T;

wherein [N1] immediately precedes [N2]-[N3].

10. The AAV particle of claim 9, wherein [N1] comprises the amino acid sequence QS, SS, or QSS.

11. The AAV particle of claim 9 or claim 10, wherein [N1]-[N2] comprises the amino acid sequence QSSYPAEV (SEQ ID NO: 96).

12. The AAV particle of any one of claims 9-11, wherein [N1]-[N2]-[N3] comprises the amino acid sequence SSYPAEVVQ (SEQ ID NO: 121) or QSSYPAEVVQK (SEQ ID NO: 150).

13. The AAV particle of any one of claims 9-12, wherein the AAV capsid variant further comprises [N0] comprising X_A, X_B, and X_C, wherein:

(a) X_A of [N0] is T, I, or N;

(b) X_B of [N0] is N; and

(c) X_C of [N0] is N, T, S, or K;

wherein [N0] immediately precedes [N1].

14. The AAV particle of claim 13, wherein [N0] comprises the amino acid sequence TN, NN, or TNN.

15. The AAV particle of claim 13 or claim 14, wherein [N0]-[N1] comprises the amino acid sequence TNNQSS (SEQ ID NO: 183).

16. The AAV particle of any one of claims 13-15, wherein [N0]-[N1]-[N2]-[N3] comprises the amino acid sequence TNNQSSYPAEVVQK (SEQ ID NO: 500).

17. The AAV particle of any one of claims 13-16, wherein the AAV capsid variant further comprises [N4] comprising X_G and X_H, wherein:

(a) X_G of [N4] is T, P, or N; and

(b) X_H of [N4] is A;

wherein [N4] is present immediately subsequent to [N3].

18. The AAV particle of claim 17, wherein [N4] comprises the amino acid sequence TA.

19. The AAV particle of claim 17 or claim 18, wherein [N0]-[N1]-[N2]-[N3]-[N4] comprises the amino acid sequence TNNQSSYPAEVVQKTA (SEQ ID NO: 1533).

20. The AAV particle of any one of claims 17-19, wherein [N0]-[N1]-[N2]-[N3]-[N4] is present in loop VIII, wherein loop VIII is present at amino acids comprising those corresponding to positions 571-599 of the amino acid sequence of SEQ ID NO: 982.

21. The AAV particle of claim 20, wherein:

(i) [N0] is present at amino acids corresponding to positions 571-573 of the amino acid sequence of SEQ ID NO: 982;

(ii) [N1] is present at amino acids corresponding to positions 574-576 of the amino acid sequence of SEQ ID NO: 982;

(iii) [N2] is present at amino acids corresponding to positions 577-581 of the amino acid sequence of SEQ ID NO: 982;

(iv) [N3] is present at amino acids corresponding to positions 582-584 of the amino acid sequence of SEQ ID NO: 982; and

(v) [N4] is present at amino acids corresponding to positions 585-586 of the amino acid sequence of SEQ ID NO: 982.

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

(i) a viral genome comprising a frataxin (FXN)-encoding sequence; and

(ii) an AAV capsid variant comprising an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 739, wherein the AAV capsid variant comprises at least 4, at least 5, at least 6, at least 7, or all 8 consecutive amino acids from the amino acid sequence YPAEVVQK (SEQ ID NO: 943).

23. The AAV particle of claim 22, wherein:

(i) the at least 4 consecutive amino acids comprise the amino acid sequence YPAE (SEQ ID NO: 21), optionally present at amino acids corresponding to positions 577-580 of the amino acid sequence of SEQ ID NO: 982;

(ii) the at least 5 consecutive amino acids comprise the amino acid sequence YPAEV (SEQ ID NO: 1), optionally present at amino acids corresponding to positions 577-581 of the amino acid sequence of SEQ ID NO: 982;

(iii) the at least 6 consecutive amino acids comprise the amino acid sequence YPAEVV (SEQ ID NO: 725), optionally present at amino acids corresponding to positions 577-582 of the amino acid sequence of SEQ ID NO: 982; or

(iv) the at least 7 consecutive amino acids comprise the amino acid sequence YPAEVVQ (SEQ ID NO: 726), optionally present at amino acids corresponding to positions 577-583 of the amino acid sequence of SEQ ID NO: 982.

24. The AAV particle of claim 22 or claim 23, wherein the AAV capsid variant comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943) present at amino acids corresponding to positions 577-584 of the amino acid sequence of SEQ ID NO: 982.

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

(i) a viral genome comprising a frataxin (FXN)-encoding sequence; and

(ii) an AAV capsid variant comprising an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO: 739, wherein the AAV capsid variant comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943) at amino acids corresponding to positions 577-584 of the amino acid sequence of SEQ ID NO: 982.

26. The AAV particle of any one of claims 1-25, wherein the AAV capsid variant further comprises

(i) an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 738; and/or

(ii) an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 982.

27. An adeno-associated virus (AAV) particle comprising an AAV capsid variant and a viral genome, wherein the viral genome comprises a frataxin (FXN)-encoding sequence and the AAV capsid variant comprises

(i) an amino acid sequence having at least 99% identity to the amino acid sequence of SEQ ID NO: 982, wherein the AAV capsid variant comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943);

(ii) an amino acid sequence having at least 99% identity to the amino acid sequence of SEQ ID NO: 738, wherein the AAV capsid variant comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943); and/or

(iii) an amino acid sequence having at least 99% identity to the amino acid sequence of SEQ ID NO: 739, wherein the AAV capsid variant comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943).

28. The AAV particle of any one of claims 1-27, wherein the AAV capsid variant comprises

(i) the amino acid sequence of SEQ ID NO: 982;

(ii) the amino acid sequence of SEQ ID NO: 738; and/or

(iii) the amino acid sequence of SEQ ID NO: 739.

29. The AAV particle of any one of claims 1-28, wherein the FXN-encoding sequence encodes a wildtype FXN protein.

30. The AAV particle of any one of claims 1-29, wherein the FXN-encoding sequence encodes a human FXN protein.

31. The AAV particle of claim 30, wherein the human FXN protein comprises the amino acid sequence of SEQ ID NO: 1825, or an amino acid 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% identical thereto, optionally wherein the human FXN protein is a wildtype human FXN protein.

32. The AAV particle of any one of claims 1-31, wherein the FXN-encoding sequence comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 1825.

33. The AAV particle of any one of claims 1-32, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is 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% identical thereto.

34. The AAV particle of any one of claims 1-33, wherein the viral genome further comprises a promoter operably linked to the FXN-encoding sequence.

35. The AAV particle of claim 34, wherein the promoter comprises a human elongation factor 1α-subunit (EF1α) promoter, a cytomegalovirus (CMV) immediate-early enhancer and/or promoter, a chicken β-actin (CBA) promoter, a CAG promoter, a β glucuronidase (GUSB) promoter, a ubiquitin C (UBC) promoter, a neuron-specific enolase (NSE) promoter, 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 Ca2+/calmodulin-dependent protein kinase II (CaMKII) promoter, a metabotropic glutamate receptor 2 (mGluR2) promoter, a neurofilament light chain (NFL) promoter, a neurofilament heavy chain (NFH) promoter, a β-globin minigene nβ2 promoter, a preproenkephalin (PPE) promoter, an enkephalin (Enk) and excitatory amino acid transporter 2 (EAAT2) promoter, a glial fibrillary acidic protein (GFAP) promoter, a myelin basic protein (MBP) promoter, a cardiovascular promoter (e.g., αMHC, cTnT, and CMV-MLC2k), a liver promoter (e.g., hAAT, TBG), a skeletal muscle promoter (e.g., desmin, MCK, C512), or a functional fragment or truncation of any of the foregoing.

36. The AAV particle of claim 34 or claim 35, wherein the promoter is a CMV promoter or CBA promoter, or a functional fragment or truncation of a CMV promoter or CBA promoter.

37. The AAV particle of claim 36, wherein the promoter is a truncated CBA promoter.

38. The AAV particle of claim 37, wherein the truncated CBA promoter is 50-400 nucleotides in length, e.g., 100-332 nucleotides in length.

39. The AAV particle of any one of claims 36-38, wherein the promoter comprises the nucleotide sequence of any one of SEQ ID NOs: 1738, 1740, and 1742 or 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%, or at least 99% identical thereto.

40. The AAV particle of claim 36, wherein the promoter is a truncated CMV promoter.

41. The AAV particle of claim 40, wherein the truncated CMV promoter is 50-300 nucleotides in length.

42. The AAV particle of claim 40 or claim 41, wherein the promoter comprises the nucleotide sequence of SEQ ID NO: 1750 or 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%, or at least 99% identical thereto.

43. The AAV particle of any one of claims 1-42, wherein the viral genome further comprises a nucleotide sequence encoding a microRNA (miR) binding site that modulates expression of the encoded FXN protein in a cell or tissue of the liver.

44. The AAV particle of claim 43, wherein the viral genome encodes 3 copies of the miR binding site.

45. The AAV particle of claim 44, wherein the 3 copies of the miR binding site are identical.

46. The AAV particle of claim 44 or claim 45, wherein the 3 copies of the miR binding site are continuous.

47. The AAV particle of any one of claims 43-46, wherein the miR binding site is a miR122 binding site, optionally wherein:

the nucleotide sequence encoding the miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1827 or a sequence having one, two, three, or at most four substitutions relative to the nucleotide sequence of SEQ ID NO: 1827; or

the viral genome encodes 3 copies of a miR122 binding site, wherein the nucleotide sequence encoding the 3 copies of the miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or a nucleotide sequence having one, two, three, four, five, six, seven, eight, nine, or at most ten substitutions relative thereto.

48. The AAV particle of any one of claims 1-47, wherein the viral genome further comprises at least one inverted terminal repeat (ITR) region.

49. The AAV particle of claim 48, wherein the at least one ITR region comprises an AAV2 ITR.

50. The AAV particle of claim 48 or claim 49, wherein the viral genome comprises a 5’ ITR region and a 3’ ITR region.

51. The AAV particle of claim 50, wherein the 5’ ITR region and 3’ ITR region are each an AAV2 ITR.

52. The AAV particle of claim 51, wherein the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 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% identical thereto; and/or the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 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% identical thereto.

53. The AAV particle of any one of claims 1-52, wherein the viral genome further comprises an intron/exon region comprising an intron region and/or an exon region.

54. The AAV particle of claim 53, wherein the intron/exon region comprises:

an immediate-early 1 (ie1) intron region and/or a human beta-globin (hBglobin) intron 2 region; and/or

an ie1 exon region and/or an hBglobin exon region.

55. The AAV particle of claim 53 or claim 54, wherein the intron region comprises:

the nucleotide sequence of SEQ ID NO: 1819 or 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%, or at least 99% identical thereto; and/or

the nucleotide sequence of SEQ ID NO: 1820 or 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%, or at least 99% identical thereto.

56. The AAV particle of any one of claims 53-55, wherein the exon region comprises:

the nucleotide sequence of SEQ ID NO: 1817 or 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%, or at least 99% identical thereto; and/or

the nucleotide sequence of SEQ ID NO: 1821 or 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%, or at least 99% identical thereto.

57. The AAV particle of any one of claims 1-56, wherein the viral genome further comprises a polyadenylation (polyA) region.

58. The AAV particle of claim 57, wherein the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or 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%, or at least 99% identical thereto.

59. The AAV particle of any one of claims 1-30, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797 or 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%, or at least 99% identical thereto.

60. The AAV particle of any one of claims 1-30, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1801 or 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%, or at least 99% identical thereto.

61. The AAV particle of any one of claims 1-30, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1808 or 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%, or at least 99% identical thereto.

62. The AAV particle of any one of claims 1-30, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1809 or 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%, or at least 99% identical thereto.

63. The AAV particle of any one of claims 1-30, wherein the viral genome comprises:

(i) a 5’ inverted terminal repeat (ITR) region;

(ii) a promoter;

(iii) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is 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% identical thereto; and

(iv) a 3’ ITR region.

64. The AAV particle of any one of claims 1-30, wherein the viral genome comprises:

(i) a 5’ inverted terminal repeat (ITR) region;

(ii) a promoter;

(iii) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is at least 80%, 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% identical thereto;

(iv) a nucleotide sequence encoding at least one miR122 binding site; and

(v) a 3’ ITR region.

65. The AAV particle of any one of claims 1-30, wherein the viral genome comprises:

(i) a 5’ inverted terminal repeat (ITR) region;

(ii) a promoter;

(iii) an intron/exon region;

(iv) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is 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% identical thereto;

(v) a nucleotide sequence encoding at least one miR122 binding site; and

(vi) a 3’ ITR region.

66. The AAV particle of any one of claims 1-30, wherein the viral genome comprises:

(i) a 5’ inverted terminal repeat (ITR) region;

(ii) a promoter;

(iii) an intron/exon region;

(iv) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is 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% identical thereto;

(v) a nucleotide sequence encoding at least one miR122 binding site;

(vi) a polyadenylation (polyA) region; and

(vii) a 3’ ITR region.

67. The AAV particle of any one of claims 1-30, wherein the viral genome comprises:

(i) a 5’ inverted terminal repeat (ITR) region;

(ii) a promoter;

(iii) an intron/exon region;

(iv) the FXN-encoding sequence, wherein the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or a nucleotide sequence that is 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% identical thereto;

(v) a nucleotide sequence encoding at least one miR122 binding site;

(vi) a polyadenylation (polyA) region;

(vii) a filler sequence; and

(viii) a 3’ ITR region.

68. The AAV particle of claim 66 or claim 67, wherein:

(i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or 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%, or at least 99% identical thereto;

(ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1742 or 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%, or at least 99% identical thereto;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or 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%, or at least 99% identical thereto;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or 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%, or at least 99% identical thereto;

(v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or 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%, or at least 99% identical thereto;

(vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or 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%, or at least 99% identical thereto; and/or

(vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or 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%, or at least 99% identical thereto.

69. The AAV particle of claim 68, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1841 or 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%, or at least 99% identical thereto, optionally wherein the filler sequence is positioned 3’ to the polyA region and 5’ to the 3’ ITR region.

70. The AAV particle of claim 66 or claim 67, wherein:

(i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or 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%, or at least 99% identical thereto;

(ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1750 or 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%, or at least 99% identical thereto;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or 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%, or at least 99% identical thereto;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or 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%, or at least 99% identical thereto;

(v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or 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%, or at least 99% identical thereto;

(vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or 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%, or at least 99% identical thereto; and/or

(vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or 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%, or at least 99% identical thereto.

71. The AAV particle of claim 70, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1840 or 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%, or at least 99% identical thereto, optionally wherein the filler sequence is positioned 3' to the polyA region and 5' to the 3' ITR region.

72. The AAV particle of claim 66 or claim 67, wherein:

(i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or 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%, or at least 99% identical thereto;

(ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1738 or 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%, or at least 99% identical thereto;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or 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%, or at least 99% identical thereto;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or 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%, or at least 99% identical thereto;

(v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or 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%, or at least 99% identical thereto;

(vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or 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%, or at least 99% identical thereto; and/or

(vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or 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%, or at least 99% identical thereto.

73. The AAV particle of claim 72, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1838 or 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%,

or at least 99% identical thereto, optionally wherein the filler sequence is positioned 3’ to the polyA region and 5’ to the 3’ ITR region.

74. The AAV particle of claim 66 or claim 67, wherein:

(i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811 or 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%, or at least 99% identical thereto;

(ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1740 or 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%, or at least 99% identical thereto;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816 or 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%, or at least 99% identical thereto;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824 or 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%, or at least 99% identical thereto;

(v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826 or 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%, or at least 99% identical thereto;

(vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828 or 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%, or at least 99% identical thereto; and/or

(vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812 or 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%, or at least 99% identical thereto.

75. The AAV particle of claim 74, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1839 or 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%, or at least 99% identical thereto, optionally wherein the filler sequence is positioned 3’ to the polyA region and 5’ to the 3’ ITR region.

76. The AAV particle of any one of claims 65-67, wherein the viral genome comprises

(a) the nucleotide sequence of SEQ ID NO: 1797 or 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%, or at least 99% identical thereto;

(b) the nucleotide sequence of SEQ ID NO: 1801 or 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%, or at least 99% identical thereto;

(c) the nucleotide sequence of SEQ ID NO: 1808 or 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%, or at least 99% identical thereto; or

(d) the nucleotide sequence of SEQ ID NO: 1809 or 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%, or at least 99% identical thereto.

77. The AAV particle of any one of claims 66-68, wherein:

(i) the 5' ITR region comprises the nucleotide sequence of SEQ ID NO: 1811;

(ii) the promoter comprises the nucleotide sequence of SEQ ID NO: 1742;

(iii) the intron/exon region comprises the nucleotide sequence of SEQ ID NO: 1816;

(iv) the FXN-encoding sequence comprises the nucleotide sequence of SEQ ID NO: 1824;

(v) the nucleotide sequence encoding the at least one miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 1826;

(vi) the polyA region comprises the nucleotide sequence of SEQ ID NO: 1828; and

(vii) the 3' ITR region comprises the nucleotide sequence of SEQ ID NO: 1812.

78. The AAV particle of claim 77, wherein the viral genome further comprises a filler sequence comprising the nucleotide sequence of SEQ ID NO: 1841, wherein the filler sequence is positioned 3’ to the polyA region and 5’ to the 3’ ITR region.

79. The AAV particle of any one of claims 65-68 or any one of claims 76-78, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

80. The AAV particle of any one of claims 65-68 or any one of claims 76-79, wherein the viral genome consists of the nucleotide sequence of SEQ ID NO: 1797.

81. An adeno-associated virus (AAV) particle comprising an AAV capsid variant and a viral genome comprising a frataxin (FXN)-encoding sequence,

wherein the AAV capsid variant comprises:

(i) the amino acid sequence of SEQ ID NO: 982 or an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto, wherein the AAV capsid variant comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943);

(ii) the amino acid sequence of SEQ ID NO: 738 or an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto, wherein the AAV capsid variant comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943); or

(iii) the amino acid sequence of SEQ ID NO: 739 or an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto, wherein the AAV capsid variant comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943); and

wherein the viral genome comprises:

(a) the nucleotide sequence of SEQ ID NO: 1797 or 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%, or at least 99% identical) thereto;

(b) the nucleotide sequence of SEQ ID NO: 1801 or 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%, or at least 99% identical thereto;

(c) the nucleotide sequence of SEQ ID NO: 1808 or 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%, or at least 99% identical thereto; or

(d) the nucleotide sequence of SEQ ID NO: 1809 or 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%, or at least 99% identical thereto.

82. An adeno-associated virus (AAV) particle comprising a viral genome comprising the nucleotide sequence of SEQ ID NO: 1824 and an AAV capsid variant comprising the amino acid sequence of SEQ ID NO: 982, the amino acid sequence of SEQ ID NO: 738, and/or the amino acid sequence of SEQ ID NO: 739.

83. The AAV particle of claim 82, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797.

84. The AAV particle of any one of claims 1-83, wherein the viral genome is single-stranded.

85. A cell comprising the AAV particle of any one of claims 1-84, optionally wherein the cell is a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell.

86. A method of making the AAV particle of any one of claims 1-84, wherein the method comprises:

(i) providing a cell comprising

the viral genome comprising a FXN-encoding sequence and

a nucleic acid encoding an AAV capsid variant; and

(ii) incubating the cell under conditions suitable to encapsulate the viral genome in the AAV capsid variant;

thereby making the AAV particle.

87. The method of claim 86, wherein the viral genome comprises

(a) the nucleotide sequence of SEQ ID NO: 1797 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;

(b) the nucleotide sequence of SEQ ID NO: 1801 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;

(c) the nucleotide sequence of SEQ ID NO: 1808 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; or

(d) the nucleotide sequence of SEQ ID NO: 1809 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; and

wherein the AAV capsid variant comprises

(i) the amino acid sequence of SEQ ID NO: 982 or an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto, wherein the AAV capsid variant comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943);

(ii) the amino acid sequence of SEQ ID NO: 738 or an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least

98%, or at least 99% identity thereto, wherein the AAV capsid variant comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943); or

(iii) the amino acid sequence of SEQ ID NO: 739 or an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto, wherein the AAV capsid variant comprises the amino acid sequence YPAEVVQK (SEQ ID NO: 943).

88. The method of claim 86, wherein the viral genome comprises

(a) the nucleotide sequence of SEQ ID NO: 1797 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;

(b) the nucleotide sequence of SEQ ID NO: 1801 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;

(c) the nucleotide sequence of SEQ ID NO: 1808 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; or

(d) the nucleotide sequence of SEQ ID NO: 1809 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; and

wherein the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 982, the amino acid sequence of SEQ ID NO: 738, and/or the amino acid sequence of SEQ ID NO: 739.

89. The method of claim 86, wherein the viral genome comprises the nucleotide sequence of SEQ ID NO: 1797 and the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 982, the amino acid sequence of SEQ ID NO: 738, and/or the amino acid sequence of SEQ ID NO: 739.

90. The method of any one of claims 86-89, further comprising, prior to step (i), introducing a nucleic acid molecule comprising the viral genome into the cell.

91. The method of any one of claims 86-90, further comprising, prior to step (i), introducing the nucleic acid encoding the AAV capsid variant into the cell.

92. The method of any one of claims 86-91, wherein the cell comprises a mammalian cell (e.g., an HEK293 cell), an insect cell (e.g., an Sf9 cell), or a bacterial cell.

93. A pharmaceutical composition comprising the AAV particle of any one of claims 1-84 and a pharmaceutically acceptable excipient.

94. An *in vitro* method of delivering an AAV particle encoding a frataxin (FXN) protein to a cell, comprising contacting the cell with an effective amount of the pharmaceutical composition of claim 93 or the AAV particle of any one of claims 1-84.

95. The method of claim 94, wherein the cell is obtained from a subject.

96. The method of claim 95, wherein the subject has, has been diagnosed with having, or is at risk of having a disorder associated with FXN deficiency, optionally wherein the disorder is Friedreich's Ataxia (FA).

97. Use of the pharmaceutical composition of claim 93 or the AAV particle of any one of claims 1-84 in the manufacture of a medicament for the treatment of a subject having or diagnosed with having a disorder associated with frataxin (FXN) deficiency.

98. The use of claim 97, wherein the subject has, has been diagnosed with having, or is at risk of having Friedreich's Ataxia (FA).

99. Use of the pharmaceutical composition of claim 93 or the AAV particle of any one of claims 1-84 in the manufacture of a medicament for the treatment of a subject having or diagnosed with having a disorder, wherein the disorder is Friedreich's Ataxia (FA).

100. The use of any one of claims 97-99, wherein the treatment results in prevention of progression of the disorder in the subject.

101. The use of any one of claims 97-100, wherein the treatment results in amelioration of at least one symptom of the disorder.

102. The use of claim 101, wherein the at least one symptom comprises impaired sensory functions, impaired motor function (e.g., ataxia and/or involuntary movements), fatigue, chronic pain, seizures, impaired speech, sleep disturbances, metabolic disorders (e.g., diabetes), and/or increased spasticity.

103. The use of any one of claims 97-102, wherein the treatment stabilizes, slows the progression of, or improves the subject's disorder as determined by the modified Friedreich Ataxia Rating Scale (mFARS), the Scale for the Assessment and Rating of Ataxia (SARA), and/or the International Cooperative Ataxia Rating Scale (ICARS).

104. The use of claim 103, wherein the treatment slows the subject's progression of the disorder as measured by mFARS, SARA, and/or ICARS relative to an individual with the disorder who has not been administered the pharmaceutical composition or the AAV particle.

105. The use of any one of claims 97-104, wherein the subject is a human.

106. The use of any one of claims 97-105, wherein the AAV particle or the pharmaceutical composition is to be delivered to a cell or tissue of the central nervous system (CNS) in the subject.

107. The use of claim 106, wherein the cell or tissue of the CNS is a cell or tissue of the spinal cord, temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje cell layer, deep cerebellar nuclei, dentate nucleus, brainstem, cerebellum, or a combination thereof.

108. The use of any one of claims 97-107, wherein the AAV particle or the pharmaceutical composition is to be delivered to the subject via intravenous administration.

109. The use of any one of claims 97-108, wherein the level of FXN expression, e.g., FXN gene expression, FXN mRNA expression, and/or FXN protein expression, in the subject, e.g., in a cell, tissue, or fluid of the subject, is to be evaluated, e.g., measured.

110. The use of claim 109, wherein the level of FXN protein expression is to be measured by an enzyme-linked immunosorbent assay (ELISA), a Western blot, an immunohistochemistry assay, or a frataxin biofluid assay.

111. The use of claim 109 or claim 110, wherein the subject's level of FXN expression is to be evaluated before and/or after administration of the pharmaceutical composition or AAV particle, optionally wherein the subject's level of FXN expression before administration is compared to the subject's level of FXN expression after administration.

112. The use of any one of claims 109-111, wherein the level of FXN expression in a cell or tissue of the CNS is to be evaluated.

113. The use of claim 112, wherein the cell or tissue of the CNS is a cell or tissue of the spinal cord, temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje cell layer, deep cerebellar nuclei, dentate nucleus, brainstem, and/or cerebellum.

114. The use of any one of claims 109-111, wherein the level of FXN expression in a peripheral cell or tissue is to be evaluated.

115. The use of claim 114, wherein the peripheral cell or tissue is a cell or tissue of the heart and/or muscle.

116. The use of any one of claims 109-115, wherein the subject's level of FXN protein expression after administration is increased relative to the subject's level of FXN protein expression before administration.

117. The use of any one of claims 97-116, wherein the level of FXN protein activity in the subject is to be evaluated, e.g., measured.

118. The use of any one of claims 97-117, wherein administration of the pharmaceutical composition or AAV particle to the subject results in an increase in:

(i) the level of FXN protein or FXN gene expression in a cell, tissue, (e.g., a cell or tissue of the CNS, e.g., spinal cord, temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje cell layer, deep cerebellar nuclei, dentate nucleus, brainstem, and/or cerebellum), and/or fluid (e.g., CSF and/or serum), of the subject relative to baseline;

(ii) the number and/or level of viral genomes (VG) per cell in a CNS tissue (e.g., spinal cord, temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje cell layer, deep cerebellar nuclei, dentate nucleus, brainstem, and/or cerebellum) of the subject relative to the number and/or level of VG per cell in a peripheral tissue of the subject; and/or

(iii) the level of FXN activity in a cell, tissue, (e.g., a cell or tissue of the CNS, e.g., spinal cord, temporal cortex, perirhinal cortex, globus pallidus, putamen, caudate, thalamus, hippocampus, geniculate nucleus, Purkinje cell layer, deep cerebellar nuclei, dentate nucleus, brainstem, and/or cerebellum) of the subject relative to baseline and/or relative to FXN activity in a cell, tissue, or fluid of an individual with a disorder associated with FXN deficiency who has not been administered the pharmaceutical composition or AAV particle.

119. The use of any one of claims 97-116, wherein at least one additional therapeutic agent and/or therapy is to be further administered to the subject.

120. The use of claim 119, wherein the at least one additional therapeutic agent and/or therapy comprises an agent and/or therapy suitable for treating a disorder associated with FXN deficiency (e.g., Friedreich's Ataxia).

121. The use of claim 119 or claim 120, wherein the at least one additional therapeutic agent and/or therapy comprises omaveloxolone or idebenone.

122. The use of any one of claims 97-121, wherein an immunosuppressant is to be further administered to the subject.

123. The use of claim 122, wherein the immunosuppressant comprises a corticosteroid (e.g., prednisone, prednisolone, methylprednisolone, and/or dexamethasone), rapamycin, mycophenolate mofetil, tacrolimus, rituximab, and/or eculizumab hydroxychloroquine.

124. The pharmaceutical composition of claim 93 or the AAV particle of any one of claims 1-84 for use for use in therapy.