CLAIMS

1. An adeno-associated viral (AAV) viral genome comprising a nucleic acid positioned between two inverted terminal repeats (ITRs), wherein said nucleic acid when expressed inhibits or suppresses the expression of a first target gene and a second target in a cell, wherein said nucleic acid sequence comprises, in a 5' to 3' order:

(i) (a) a first 5' flanking region, a nucleotide sequence encoding a first sense strand sequence, a first loop region, a nucleotide sequence encoding a first antisense strand sequence, and a first 3' flanking region; or

(b) a first 5' flanking region, a nucleotide sequence encoding a first antisense strand sequence, a first loop region, a nucleotide sequence encoding a first sense strand sequence, and a first 3' flanking region;

wherein the first antisense strand is complementary to an mRNA of the first target gene; and

(ii) (a) a second 5' flanking region, a nucleotide sequence encoding a second sense strand sequence, a second loop region, and a nucleotide sequence encoding a second antisense strand sequence; or

(b) a second 5' flanking region, a nucleotide sequence encoding a second antisense strand sequence, a second loop region, a nucleotide sequence encoding a second sense strand sequence, and a second 3' flanking region;

wherein the second antisense strand is complementary to an mRNA of the second target gene;

wherein:

the first 5' flanking region comprises the nucleotide sequence of any one of SEQ ID NOs: 1503-1509, 1692, or 1782; and the second 5' flanking region comprises the nucleotide sequence of any one of SEQ ID NOs: 1503-1509, 1692, or 1782;

the first loop region comprises the nucleotide sequence of any one of SEQ ID NOs: 1510-1517, or 1693-1694; and the second loop region comprises the nucleotide sequence of any one of SEQ ID NOs: 1510-1517, or 1693-1694; and

the first 3' flanking region comprises the nucleotide sequence of any one of SEQ ID NOs: 1518-1522, 1695, or 1783; and the second 3' flanking region comprises the nucleotide sequence of any one of SEQ ID NOs: 1518-1522, 1695, or 1783.

2. The AAV genome of claim 1, wherein:

(i) the first sense strand sequence and the first antisense strand sequence share a region of complementarity of at least 12 nucleotides in length; and

(ii) the second sense strand sequence and the second antisense strand sequence share a region of complementarity of 12 nucleotides in length

3. The AAV viral genome of claim 1 or 2, wherein the first target gene is the same as the second target gene.

4. The AAV genome of claim 1 or 2, wherein the first target gene is not the same as the second target gene.

5. The AAV viral genome of any one of claims 1-4, wherein the first target gene Huntingtin (HTT) or SOD1.

6. The AAV viral genome of any one of claims 1-5, wherein the second target gene is HTT or SOD1.

7. The AAV viral genome of any one of claims 1-6, wherein the first target gene and the second target gene is HTT.

8. The AAV viral genome of any one of claims 1-6, wherein the first target gene and the second target gene is SOD1.

9. The AAV viral genome of any one of claims 1-5, wherein the first target gene is HTT and the second target gene is SOD1.

10. The AAV viral genome of any one of claims 1-5, wherein the first target gene is SOD1 and the second target gene is HTT.

11. The AAV viral genome of any one of claims 1-10, wherein:

(i) the first sense strand sequence and the second sense strand sequence are, each independently, 19 to 24 nucleotides in length or 19 to 21 nucleotides in length; or

(ii) the first sense strand sequence and the second sense strand sequence are, each independently, 20 nucleotides in length, 21 nucleotides in length, or 22 nucleotides in length.

12. The AAV viral genome of any one of claims 1-11, wherein:

(i) one or both of the first sense strand sequence and the first antisense strand sequence comprise a 3' overhang of at least 1 or 2 nucleotides; and/or

(ii) one or both of the second sense strand sequence and the second antisense strand sequence comprise a 3' overhang of at least 1 or 2 nucleotides.

13. The AAV viral genome of any one of claims 1-12, wherein:

(i) the first 5' flanking region comprises the nucleotide sequence of SEQ ID NO: 1504, the first loop region comprises the nucleotide sequence of SEQ ID NO: 1511, and the first 3' flanking region comprises the nucleotide sequence of SEQ ID NO: 1518; and the second 5' flanking region comprises the nucleotide sequence of SEQ ID NO: 1505, the second loop region comprises the nucleotide sequence of SEQ ID NO: 1513, and the second 3' flanking region comprises the nucleotide sequence of SEQ ID NO: 1520; or

(ii) the first 5' flanking region comprises the nucleotide sequence of SEQ ID NO: 1505, the first loop region comprises the nucleotide sequence of SEQ ID NO: 1513, and the first 3' flanking region comprises the nucleotide sequence of SEQ ID NO: 1520; and the second 5' flanking region comprises the nucleotide sequence of SEQ ID NO: 1504, the second loop region comprises the nucleotide sequence of SEQ ID NO: 1511, and the second 3' flanking region comprises the nucleotide sequence of SEQ ID NO: 1518.

14. The AAV viral genome of any one of claims 1-13, which further comprises:

(i) a promoter present 5' relative to the first 5' flanking region; or

(ii) a first promoter present 5' relative to the first 5' flanking region and a second promoter present 5' relative to the second 5' flanking region.

15. The AAV viral genome of claim 14, wherein the first promoter and/or the second promoter is a ubiquitous promoter.

16. The AAV viral genome of claim 15, wherein the first promoter and/or the second promoter is a cell-type specific promoter.

17. The AAV viral genome of any one of claims 14-16, wherein the first promoter and/or the second promoter is a CBA promoter, a CMV promoter, a PGK promoter, an H1 promoter, a UBC promoter, a GUSB promoter, an NSE promoter, a synapsin promoter, a MeCP2 promoter, or a GFAP promoter.

18. The AAV viral genome of any one of claims 1-17, which further comprises:

(i) an intron;

(ii) a filler sequence; and/or

(iii) a polyadenylation (polyA) sequence.

19. An adeno-associated virus (AAV) particle comprising the AAV viral genome of any one of claims 1-18, and an AAV capsid protein.

20. The AAV particle of claim 19, wherein the AAV capsid protein is an AAV9 capsid protein or a variant thereof or an AAV5 capsid protein or a variant thereof.

21. A cell comprising the AAV viral genome of any one of claims 1-18, or the AAV particle of claim 19 or 20, which is a mammalian cell, an HEK293 cell, an insect cell, an Sf9 cell, a cell of the central nervous system, a neuron, a medium spiny neuron, a motor neuron, or an astrocyte.

22. A pharmaceutical composition comprising an AAV particle comprising the AAV viral genome of any one of claims 1-18 or the AAV particle of claim 19 or 20, and a pharmaceutically acceptable excipient.

23. An AAV particle comprising the AAV viral genome of any one of claims 1-18, the AAV particle of claim 19 or 20, or the pharmaceutical composition of claim 22, for use in inhibiting the expression of a first target gene and/or a second target gene in a cell.

24. Use of an AAV particle comprising the AAV viral genome of any one of claims 1-18, the AAV particle of claim 19 or 20, or the pharmaceutical composition of claim 22, in the manufacture of a medicament for inhibiting the expression of a first target gene and/or a second target gene in a cell.

25. The AAV particle or pharmaceutical composition for use of claim 23 or the use of claim 24, wherein the cell is a medium spiny neuron, a cortical neuron, a motor neuron, or an astrocyte.

26. The AAV particle or pharmaceutical composition for use of claim 23 or 25 or the use of claim 24 or 25, wherein the first target gene and/or the second target gene is expressed in a neurologic cell, tissue, or organ.

27. The AAV particle or pharmaceutical composition for use of any one of claims 23, 25, or 26 or the use of any one of claims 24-26, wherein the first target gene and/or the second target gene is HTT or SOD1.

28. The AAV particle or pharmaceutical composition for use of any one of claims 23, 25, or 26 or the use of any one of claims 24-26, wherein the cell is in a subject.

29. The AAV particle or pharmaceutical composition for use of claim 28 or the use of claim 28, wherein the subject has a disease of the central nervous system (CNS).

30. An AAV particle comprising the AAV viral genome of any one of claims 1-18, the AAV particle of claim 19 or 20, or the pharmaceutical composition of claim 22, for use in treating a disease of the central nervous system (CNS) in a subject.

31. Use of an AAV particle comprising the AAV viral genome of any one of claims 1-18, the AAV particle of claim 19 or 20, or the pharmaceutical composition of claim 22, in the manufacture of a medicament for treating a disease of the central nervous system (CNS) in a subject.

32. The AAV particle or pharmaceutical composition for use of claim 29 or 30 or the use of claim 29 or 31, wherein disease of the CNS is Huntington's Disease (HD) or amyotrophic lateral sclerosis (ALS).

33. The AAV particle or pharmaceutical composition for use of any one of claims 23, 25, 26-30, or 32 or the use of any one of claims 24-29, 31, or 32, wherein the AAV particle or pharmaceutical composition is formulated for intravenous administration, intracisternal injection, intravascular administration, intraventricular administration, or a combination thereof.

34. A method of producing an adeno-associated virus (AAV) particle, comprising providing a cell with a polynucleotide comprising the AAV viral genome of any one of claims 1-18, at least one polynucleotide encoding AAV rep genes, and at least one polynucleotide encoding AAV cap genes; and harvesting the AAV particle from the cell, wherein the cell is a bacterial cell, a mammalian cell, or an insect cell.