CLAIMS

We claim:

1. An adeno-associated virus (AAV) viral ~~(AAV) vector~~genome comprising a ~~first~~ nucleic acid sequence ~~comprising two~~ positioned between a first inverted terminal ~~repeats (ITRs), a promoter, an intron~~repeat (ITR) and a second ~~nucleic acid sequence~~ITR, wherein said ~~second nucleic acid sequence when expressed inhibits or suppresses expression of SOD1 in~~nucleotide sequence encodes a ~~cell~~modulatory polynucleotide, wherein ~~said second nucleic acid sequence~~the modulatory polynucleotide comprises ~~a sense strand sequence and an antisense strand sequence, wherein the sense strand sequence comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides~~ from 5′ to 3′:

(a) a 5′ flanking region, wherein the nucleotide sequence ~~of sequences listed in Table 3 and~~encoding the ~~antisense strand~~5′ flanking region comprises a nucleotide sequence ~~comprises~~ selected from SEQ ID NOs: 1255-1263;

(b) an antisense sequence at least ~~15 contiguous nucleotides differing by no more than 3 nucleotides from~~ 80% identical to a nucleotide sequence selected from SEQ ID NOs: 916-1084;

(c) a loop region, wherein the nucleotide sequence ~~of sequences listed in Table 2 and wherein said sense strand sequence and antisense strand sequence share a region of complementarity of at least four nucleotides in length.~~encoding the loop region comprises a nucleotide sequence selected from SEQ ID NOs: 1264-1273;

(d) a sense strand sequence at least 80% identical to a nucleotide sequence selected from SEQ ID NOs: 1085-1253, wherein the sense strand sequence is complementary to the antisense strand sequence; and

(e) a 3′ flanking region, wherein the nucleotide sequence encoding the 3′ flanking region comprises a nucleotide sequence selected from SEQ ID NOs: 1274-1280.

2. The AAV ~~vector~~viral genome of claim 1, wherein ~~the promoter is selected from the group consisting of CMV, CBA and H1.~~:

~~3. The AAV vector of claim 2, wherein~~(a) the ~~intron is beta-globin.~~

~~4. The AAV vector of claim 3, wherein the promoter is CMV.~~

nucleotide sequence encoding the 5~~. The AAV vector of claim 3, wherein the promoter is CBA.~~

~~6. The AAV vector of claim 3, wherein the promoter is H1.~~

~~7. The AAV vector of claim 1, wherein the AAV vector~~′ flanking region comprises ~~a capsid serotype selected from the group consisting 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/r11.64, 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, AAAV, 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, AAVF9/HSC9, AAV-PHP.B, AAV-PHP.A, G2B-26, G2B-13, TH1.1-32, TH1.1-35, AAVPHP.B2, AAVPHP.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, AAVG2B4, AAVG2B5 and variants thereof.~~SEQ ID NO: 1259;

(b) the nucleotide sequence encoding the loop region comprises SEQ ID NO: 1268;

(c) the nucleotide sequence encoding the 3′ flanking region comprises SEQ ID NO: 1277; and

(d) the antisense strand sequence is at least 80% identical to the nucleotide sequence of SEQ ID NO: 963, and the sense strand sequence is at least 80% identical to the nucleotide sequence of SEQ ID NO: 1132.

3. The AAV viral genome of claim 1, wherein the antisense strand sequence comprises nucleotides 1-19 of SEQ ID NO: 963, and the sense strand sequence comprises nucleotides 1-18 of SEQ ID NO: 1132.

4. The AAV viral genome of claim 1, wherein the antisense strand sequence is at least 85% identical to SEQ ID NO: 963, and the sense strand sequence is at least 85% identical to SEQ ID NO: 1132.

5. The AAV viral genome of claim 1, wherein the sense strand sequence and/or antisense strand sequence are, independently, between 20-22 nucleotides in length.

6. The AAV viral genome of claim 1, wherein the sense strand sequence and the antisense strand sequence comprise a 3′ overhang of 1-2 nucleotides.

7. An AAV viral genome, comprising a nucleic acid sequence positioned between a first inverted terminal repeat (ITR) and a second ITR, wherein said nucleotide sequence encodes a modulatory polynucleotide, wherein the modulatory polynucleotide comprises from 5′ to 3′:

(i) a 5′ flanking region, wherein the nucleotide sequence encoding the 5′ flanking region comprises SEQ ID NO: 1259;

(ii) a sense strand sequence, which comprises nucleotides 1-18 of SEQ ID NO: 1132;

(iii) a loop region, wherein the nucleotide sequence encoding the loop region comprises SEQ ID NO: 1268;

(iv) an antisense strand sequence, which comprises nucleotides 1-19 of SEQ ID NO: 963; and

(v) a 3′ flanking region, wherein the nucleotide sequence encoding the 3′ flanking region comprises SEQ ID NO: 1277.

8. The AAV viral genome of claim 7, wherein:

(i) the first ITR comprises a nucleotide sequence selected from SEQ ID NOs: 1370-1373, and is positioned 5′ relative to the nucleotide sequence encoding the modulatory polynucleotide; and

(ii) the second ITR comprises a nucleotide sequence selected from SEQ ID NOs: 1370-1372, and is positioned 3′ relative to the nucleotide sequence encoding the modulatory polynucleotide.

9. The AAV viral genome of claim 7, wherein the first ITR comprises the nucleotide sequence of SEQ ID NO: 1371 and is positioned 5′ relative to the nucleotide sequence encoding the modulatory polynucleotide, and the second ITR comprises the nucleotide sequence of SEQ ID

NO: 1373, and is positioned 3′ relative to the nucleotide sequence encoding the modulatory polynucleotide.

10. A ~~method~~recombinant adeno-associated virus (AAV) particle comprising the AAV viral genome of claim 7, and an AAV capsid, wherein the AAV capsid comprises an AAV9 capsid or variant thereof, an AAV5 capsid or variant thereof, or an AAVrh10 capsid or variant thereof.

11. A pharmaceutical composition comprising the recombinant AAV particle of claim 10, and a pharmaceutically acceptable excipient.

12. Use of the pharmaceutical composition of claim 11 in the manufacture of a medicament for inhibiting or suppressing the expression of SOD1 gene in a cell ~~comprising administering~~, wherein the medicament is to be administered to the cell ~~a composition comprising an AAV vector of any one of claims 1-7~~.

13.

~~9.~~ The ~~method~~use of claim ~~8~~12, wherein the cell is:
(i) a mammalian cell~~.~~

~~10. The method of claim 9, wherein the mammalian cell is~~ , a motor neuron~~.~~

~~11. The method of claim 9, wherein the mammalian cell is~~ , or an astrocyte~~.~~; and/or

~~12. A method for treating and/or ameliorating~~(ii) in a subject, wherein the subject has amyotrophic lateral sclerosis (ALS~~) in a subject in need of~~).

14. The use of claim 12, wherein the SOD1 gene comprises a wild type SOD1 gene, a mutated SOD1 gene, or a combination thereof.

15. Use of the pharmaceutical composition of claim 11 in the manufacture of a medicament for the treatment~~, the method comprising administering to the~~ of amyotrophic lateral sclerosis (ALS) in a subject, wherein a therapeutically effective amount of ~~a composition~~ the medicament is to be administered to the subject.

16. The use of claim 15, wherein the ALS is:
(i) familial ALS;
(ii) sporadic ALS;
(iii) early stage ALS;
(iv) middle stage ALS; and/or
(v) late stage ALS.

17. A modulatory polynucleotide targeting a SOD1 gene comprising ~~an AAV vector of any one of claims 1-7.~~from 5′ to 3′:

(i) a 5′ flanking region, wherein the nucleotide sequence encoding the 5′ flanking region comprises a nucleotide sequence selected from SEQ ID NOs: 1255-1263;

(ii) an antisense strand at least 80% identical to a nucleotide sequence selected from SEQ ID NOs: 916-1084;

(iii) a loop region, wherein the nucleotide sequence encoding the loop region comprises a nucleotide sequence selected from SEQ ID NOs: 1264-1273;

(iv) a sense strand at least 80% identical to a nucleotide sequence selected from SEQ ID NOs: 1085-1253, wherein the sense strand sequence is complementary to the antisense strand sequence; and

(v) a 3′ flanking region, wherein the nucleotide sequence encoding the 3′ flanking region comprises a nucleotide sequence selected from SEQ ID NOs: 1274-1280.

18. A modulatory polynucleotide targeting a

~~13. The method of claim 12, wherein the expression of SOD1 is inhibited or suppressed.~~

~~14.~~ SOD1 gene comprising from 5′ to 3′:

(i) a 5′ flanking region, wherein the nucleotide sequence encoding the 5′ flanking region comprises the nucleotide sequence of SEQ ID NO: 1259;

(ii) an antisense strand sequence at least 80% identical to the nucleotide sequence of SEQ ID NO: 963;

(iii) a loop region, wherein the nucleotide sequence encoding the loop region comprises the nucleotide sequence of SEQ ID NO: 1268;

(iv) a sense strand sequence at least 80% identical to the nucleotide sequence of SEQ ID NO: 1132, wherein the sense strand sequence is complementary to the antisense strand sequence; and

(v) a 3′ flanking region, wherein the nucleotide sequence encoding the 3′ flanking region comprises the nucleotide sequence of SEQ ID NO: 1277.

19. An adeno-associated virus (AAV) viral genome comprising a nucleic acid sequence positioned between a first inverted terminal repeat (ITR) and a second ITR, wherein said nucleotide sequence encodes the modulatory polynucleotide of claim 18.

20. The AAV viral genome of claim 19, further comprising:

(i) a promoter;

(ii) an enhancer;

(iii) an intron; and/or

(iv) a poly A sequence region.

21. ~~The method of claim 13, wherein the SOD1 is wild type SOD1, mutated SOD1 with at least one mutation or both wild type SOD1 and mutated SOD1 with at least one mutation.~~

~~15. The method of claim 12, wherein the expression of SOD1 is inhibited or suppressed by about 20% to about 100%.~~

~~16. The method of claim 12, wherein the ALS is familial ALS with an identified SOD1 gene mutation.~~

~~17. The method~~The AAV viral genome of claim 20, wherein the promoter is a CMV promoter, a CBA promoter, a EF-1α promoter, a PGK promoter, a synapsin promoter, an H1 promoter, or a truncation thereof.

22. The AAV viral genome of claim 20, wherein the poly A sequence region comprises the nucleotide sequence of SEQ ID NO: 1410.

23. The AAV viral genome of claim ~~12, wherein the ALS is sporadic ALS.~~7, further comprising:

~~18. A method for inhibiting the expression of SOD1 gene in a cell wherein SOD1 gene embraces a mutation that causes a gain of function effect inside the cell, comprising administering the cell a composition comprising an AAV vector of any one of claims 1-7.~~

~~19. The method of claim 18, wherein the cell is a mammalian cell.~~

~~20. The method of claim 19, wherein the mammalian cell is a motor neuron.~~

(i) a promoter;
(ii) an enhancer;
(iii) an intron; and/or
(iv) a poly A sequence region.

24. The AAV viral genome of claim 23, wherein the promoter is a CMV promoter, a CBA promoter, a EF-1α promoter, a PGK promoter, a synapsin promoter, an H1 promoter, or a truncation thereof.

25. The AAV viral genome of claim 23, wherein the poly A sequence region comprises the nucleotide sequence of SEQ ID NO: 1410.

26. The use of claim 15, wherein the medicament is to be administered to the subject via intravenous administration, intracisternal magna administration, or combination thereof.

27. The modulatory polynucleotide of claim 18, wherein the antisense strand sequence is at least 85% identical to SEQ ID NO: 963, and the sense strand sequence is at least 85% identical to SEQ ID NO: 1132.

28. The modulatory polynucleotide of claim 18, wherein the antisense strand sequence comprises nucleotides 1-19 of SEQ ID NO: 963, and the sense strand sequence comprises nucleotides 1-18 of SEQ ID NO: 1132.

29. The pharmaceutical composition of claim 11 for use in therapy.

30. The AAV particle of claim 10 for use in therapy.

~~21. The method of claim 19, wherein the mammalian cell is an astrocyte.~~