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(54) Title: AAV CAPSID VARIANTS AND USES THEREOF

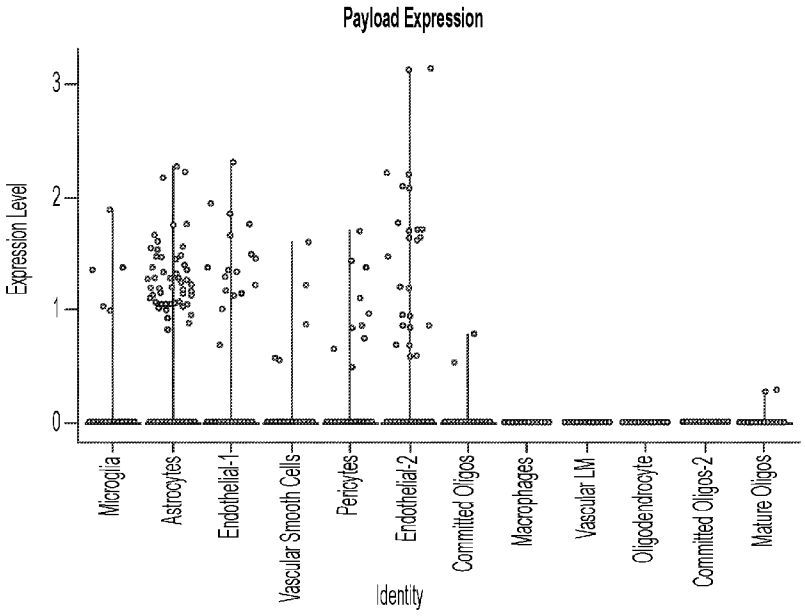

FIG. 1A

(57) Abstract: The disclosure relates to compositions and methods for the preparation, use, and/or formulation of adeno-associated virus capsid protein variants.

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AAV CAPSID VARIANTS AND USES THEREOF

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/462,007 filed on April 26, 2023; the entire contents of which are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing file, entitled V2071-3014PCT_SL.xml, was created on April 15, 2024, and is 1,786,558 bytes in size. The information in electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

[0003] The disclosure relates to compositions and methods for the preparation, use, and/or formulation of adeno-associated virus capsid proteins and variants thereof.

BACKGROUND

[0004] Gene delivery to the adult central nervous system (CNS) remains a significant challenge in gene therapy. Engineered adeno-associated virus (AAV) capsids with improved brain tropism represent an attractive solution to the limitations of CNS delivery.

[0005] AAV-derived vectors are promising tools for clinical gene transfer because of their non-pathogenic nature, their low immunogenic profile, low rate of integration into the host genome and long-term transgene expression in non-dividing cells. However, the transduction efficiency of AAV natural variants in certain organs is too low for clinical applications, and capsid neutralization by pre-existing neutralizing antibodies may prevent treatment of a large proportion of patients. For these reasons, considerable efforts have been devoted to obtaining capsid variants with enhanced properties. Of many approaches tested so far, significant advances have resulted from directed evolution of AAV capsids using *in vitro* or *in vivo* selection of capsid variants created by capsid sequence randomization using either error-prone PCR, shuffling of various parent serotypes, or insertion of fully randomized short peptides at defined positions.

[0006] Attempts at providing AAV capsids with improved properties, e.g., improved tropism to a target cell or tissue upon systemic administration, have met with limited success. As such, there is a need for improved methods of producing AAV capsids and resulting AAV capsids for delivery of a payload of interest to a target cell or tissue, e.g., a CNS cell or tissue, or a muscle cell or tissue.

SUMMARY OF THE DISCLOSURE

[0007] The present disclosure pertains at least in part, to compositions and methods for the production and use of an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid

variant. In some embodiments, the AAV capsid variant has an enhanced tropism for a tissue or a cell, e.g., a CNS tissue or a CNS cell. Said tropism can be useful for delivery of a payload, e.g., a payload described herein to a cell or tissue, for the treatment of a disorder, e.g., a neurological or a neurodegenerative disorder, a muscular or a neuromuscular disorder, or a neuro-oncological disorder.

[0008] Accordingly, in one aspect, the present disclosure provides an AAV capsid variant, comprising an amino acid sequence comprising SPH, wherein the SPH is present at a position, e.g., a position in loop IV, other than positions 456-458, numbered according to SEQ ID NO: 981, 982, or 138. In some embodiments the SPH is present at positions 454-456, 455-457, 457-459, 458-460, or 459-461, numbered according to SEQ ID NO: 981, 982, or 138.

[0009] In yet another aspect, the present disclosure provides an AAV capsid variant comprising an amino acid sequence comprising SPH, wherein the SPH is present at positions 454-456, 455-457, 457-459, 458-460, or 459-461, numbered according to SEQ ID NO: 981, 982, or 138.

[0010] In yet another aspect, the present disclosure provides an AAV capsid variant comprising an amino acid sequence having the following formula: $X_1X_2SPHX_3$, wherein X_3 does not comprise a basic amino acid, e.g., is not K or R; optionally wherein the amino acid sequence is present in loop IV. In some embodiments, loop IV comprises positions 449-460, numbered according to SEQ ID NO: 138). In some embodiments, X_3 is not K or R. In some embodiments, X_3 is P, Y, G, S, W, T, A, N, L, Q, M, I, V, or H.

[0011] In yet another aspect, the present disclosure provides an AAV capsid variant comprising an amino acid sequence having the following formula: $X_1X_2SPHX_3$, wherein X_3 is P, Y, G, S, W, T, A, N, L, Q, M, I, V, or H; optionally wherein the amino acid sequence is present in loop IV. In some embodiments, loop IV comprises positions 449-460, numbered according to SEQ ID NO: 138).

[0012] In another aspect, the present disclosure provides an AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1100-1658. In some embodiments, the amino acid sequence is present in loop IV. In some embodiments, loop IV is present at positions 449-460 numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138 or 982. In some embodiments, the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 982 or 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138 or 981. In some embodiments, the amino acid sequence. In some embodiments, the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 138 or 981.

[0013] In yet another aspect, the present disclosure provides an AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, 1379, 1382, 1384, 1387, 1390, 1391, 1396, 1406, 1407, 1409, 1410, 1411, 1423, 1427, 1431, 1434, 1440-1571, or 1573-1658, wherein the

amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138 or 982.

[0014] In yet another aspect, the present disclosure provides an AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1379, 1387, 1391, 1396, 1406, 1409, 1410, 1431, 1440-1444, 1446-1461, 1463-1470, 1472, 1474, 1475, 1477-1479, 1481-1484, 1488-1491, 1494, 1497-1499, 1514, 1515, 1520, 1521, 1529, 1540, 1544, 1546, 1548, 1550, or 1556, wherein the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138 or 982.

[0015] In yet another aspect, the present disclosure provides an AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1100-1439, wherein the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138 or 981.

[0016] In yet another aspect, the present disclosure provides an AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1100-1283, wherein the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138 or 981.

[0017] In yet another aspect, the present disclosure provides an AAV particle comprising an AAV capsid variant, described herein. In some embodiments, the AAV particle comprises a nucleic acid sequence encoding a payload. In some embodiments, the AAV particle further comprises a viral genome comprising a promoter operably linked to the nucleic acid encoding the payload.

[0018] In yet another aspect, the present disclosure provides a method of making an AAV particle comprising an AAV capsid variant described herein. The method comprises providing a host cell comprising a viral genome and incubating the host cell under conditions suitable to enclose the viral genome in the AAV capsid variant, e.g., an AAV capsid variant described herein, thereby making the AAV particle.

[0019] In yet another aspect, the present disclosure provides a method of delivering a payload to a cell or tissue (e.g., a CNS cell, a CNS tissue, a liver cell, or a liver tissue). The method comprising administering an effective amount of an AAV particle comprising an AAV capsid variant described herein.

[0020] In yet another aspect, the present disclosure provides a method of treating a subject having or diagnosed with having a genetic disorder, e.g., a monogenic disorder or a polygenic disorder. The method comprising administering to the subject an effective amount an AAV particle comprising an AAV capsid variant described herein.

[0021] In yet another aspect, the present disclosure provides a method of treating a subject having or diagnosed with having neurological, e.g., a neurodegenerative, disorder. The method comprising administering an effective amount of an AAV particle comprising an AAV capsid variant described herein.

[0022] In yet another aspect, the present disclosure provides a method of treating a subject having or diagnosed with having a neuro-oncological disorder. The method comprising administering an effective amount of an AAV particle comprising an AAV capsid variant described herein.

[0023] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following enumerated embodiments.

Enumerated Embodiments

1. An AAV capsid variant, comprising an amino acid sequence comprising SPH, wherein the SPH is present at a position, e.g., a position in loop IV, other than positions 456-458, numbered according to SEQ ID NO: 981, 982, or 138.

2. An AAV capsid variant comprising an amino acid sequence comprising SPH, wherein the SPH is present at positions 454-456, 455-457, 457-459, 458-460, or 459-461, numbered according to SEQ ID NO: 138, 981, or 982.

3. The AAV capsid variant of embodiment 1 or 2, wherein the SPH is present at positions 454-456, numbered according to SEQ ID NO: 138, 981, or 982.

4. The AAV capsid variant of embodiment 1 or 2, wherein the SPH is present at positions 455-457, numbered according to SEQ ID NO: 138, 981, or 982.

5. The AAV capsid variant of embodiment 1 or 2, wherein the SPH is present at positions 457-459, numbered according to SEQ ID NO: 138, 981, or 982.

6. The AAV capsid variant of embodiment 1 or 2, wherein the SPH is present at positions 458-460, numbered according to SEQ ID NO: 138, 981, or 982.

7. The AAV capsid variant of embodiment 1 or 2, wherein the SPH is present at positions 459-461, numbered according to SEQ ID NO: 138, 981, or 982.

8. The AAV capsid variant of any one of embodiments 1-7, which comprises an amino acid sequence comprising at least 4, 5, or 6 consecutive amino acids from any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1284-1439, or 1567-1658.

9. The AAV capsid variant of any one of embodiments 1-8, which comprises an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, relative to any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1284-1439, 1567-1658.

10. The AAV capsid variant of any one of embodiments 1-9, which comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1284-1439, 1567-1658.

11. The AAV capsid variant of any one of embodiments 1-10, which comprises the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1284-1439, 1567-1658.

12. The AAV capsid variant of any one of the preceding embodiments, wherein the amino acid sequence is present in loop IV, optionally wherein loop IV comprises positions 449-460, numbered according to SEQ ID NO: 138.

13. The AAV capsid variant of any one of embodiments 1-12, wherein the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138 or 981.

14. The AAV capsid variant of any one of embodiments 1-13, wherein the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 138 or 981.

15. The AAV capsid variant of any one of embodiments 1-14, wherein the amino acid sequence comprises any one of SEQ ID NOs: 1284-1439, and wherein the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138 or 981.

16. The AAV capsid variant of any one of embodiments 1-15, wherein the amino acid sequence comprises any one of SEQ ID NOs: 1284-1439, and wherein the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 138 or 981.

17. The AAV capsid variant of any one of embodiments 1-12, wherein the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138 or 982.

18. The AAV capsid variant of any one of embodiments 1-12 or 17, wherein the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 138 or 982.

19. The AAV capsid variant of any one of embodiments 1-12, 17, or 18, which comprises the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, 1567-1658, and wherein the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138 or 982.

20. The AAV capsid variant of any one of embodiments 1-12 or 17-19, which comprises the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, 1567-1571, 1573-1658, and wherein the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 138 or 982.

21. An AAV capsid variant comprising an amino acid sequence having the following formula: $X_1X_2SPHX_3$, wherein X_3 does not comprise a basic amino acid, e.g., is not K or R; optionally wherein the amino acid sequence is present in loop IV (e.g., wherein loop IV comprises positions 449-460, numbered according to SEQ ID NO: 138).

22. The AAV capsid variant of embodiment 21, wherein X_3 is not K or R.

23. The AAV capsid variant of embodiment 21 or 22, wherein X_3 is P, Y, G, S, W, T, A, N, L, Q, M, I, V, or H.

24. An AAV capsid variant comprising an amino acid sequence having the following formula: $X_1X_2SPHX_3$, wherein X_3 is P, Y, G, S, W, T, A, N, L, Q, M, I, V, or H; optionally wherein the amino acid sequence is present in loop IV (e.g., wherein loop IV comprises positions 449-460, numbered according to SEQ ID NO: 138).

25. The AAV capsid variant of any one of embodiments 21-24, wherein:

(i) X_1 is R, W, A, L, T, S, P, H, V, G, M, Q, I, Y, or K; and/or

(ii) X_2 is S, M, T, G, A, L, N, R, F, Y, Q, V, K, I, or H.

26. The AAV capsid variant of any one of embodiments 21-25, wherein X_1X_2 comprises RS, RM, RT, WG, RA, RL, RN, AR, RF, LG, TT, SR, RG, WT, PF, HR, VY, PR, LR, WR, VR, RQ, GT, SV, PL, ST, TL, MS, QR, AK, AL, IR, RV, RI, YR, TR, RY, GA, IT, GH, SK, KL.

27. The AAV capsid variant of any one of embodiments 21-26, wherein the amino acid sequence comprises SPHP (SEQ ID NO: 3) , SPHY (SEQ ID NO: 4), SPHG (SEQ ID NO: 5), SPHS (SEQ ID NO: 4700), SPHW (SEQ ID NO: 6), SPHT (SEQ ID NO: 7), SPHA (SEQ ID NO: 8), SPHN (SEQ ID

NO: 9), SPHL (SEQ ID NO: 10), SPHQ (SEQ ID NO: 12), SPHM (SEQ ID NO: 13), SPHI (SEQ ID NO: 14), SPHV (SEQ ID NO: 15), or SPHH (SEQ ID NO: 16).

28. The AAV capsid variant of any one of embodiments 21-27, wherein the amino acid sequence comprises SSPH (SEQ ID NO: 17), MSPH (SEQ ID NO: 18), TSPH (SEQ ID NO: 19), GSPH (SEQ ID NO: 20), ASPH (SEQ ID NO: 21), LSPH (SEQ ID NO: 22), NSPH (SEQ ID NO: 23), RSPH (SEQ ID NO: 24), FSPH (SEQ ID NO: 25), YSPH (SEQ ID NO: 26), QSPH (SEQ ID NO: 27), VSPH (SEQ ID NO: 28), KSPH (SEQ ID NO: 29), ISPH (SEQ ID NO: 30), or HSPH (SEQ ID NO: 31).

29. The AAV capsid variant of any one of embodiments 21-28, wherein the amino acid sequence comprises RSSPH (SEQ ID NO: 32), RMSPH (SEQ ID NO: 33), RTSPH (SEQ ID NO: 34), WGSPH (SEQ ID NO: 35), RASPH (SEQ ID NO: 37), RLSPH (SEQ ID NO: 38), RNSPH (SEQ ID NO: 40), ARSPH (SEQ ID NO: 41), RFSPH (SEQ ID NO: 42), LGSPH (SEQ ID NO: 43), TTSPH (SEQ ID NO: 44), SRSPH (SEQ ID NO: 45), RGSPH (SEQ ID NO: 46), WTSPH (SEQ ID NO: 47), PFSPH (SEQ ID NO: 48), HRSPH (SEQ ID NO: 49), VYSPH (SEQ ID NO: 50), PRSPH (SEQ ID NO: 53), LRSPH (SEQ ID NO: 54), WRSPH (SEQ ID NO: 55), VRSPH (SEQ ID NO: 56), RQSPH (SEQ ID NO: 57), GTSPH (SEQ ID NO: 58), SVSPH (SEQ ID NO: 59), PLSPH (SEQ ID NO: 60), STSPH (SEQ ID NO: 61), TLSPH (SEQ ID NO: 62), MSSPH (SEQ ID NO: 63), QRSPH (SEQ ID NO: 64), AKSPH (SEQ ID NO: 65), ALSPH (SEQ ID NO: 66), IRSPH (SEQ ID NO: 67), RVSPH (SEQ ID NO: 68), RISPH (SEQ ID NO: 69), YRSPH (SEQ ID NO: 70), TRSPH (SEQ ID NO: 71), RYSPH (SEQ ID NO: 72), GASPH (SEQ ID NO: 73), ITSPH (SEQ ID NO: 74), GHSPH (SEQ ID NO: 75), SKSPH (SEQ ID NO: 76), or KLSPH (SEQ ID NO: 77).

30. The AAV capsid variant of any one of embodiments 21-29, wherein the amino acid sequence comprises SSPHP (SEQ ID NO: 78), SSPHY (SEQ ID NO: 79), MSPHG (SEQ ID NO: 80), MSPHP (SEQ ID NO: 81), TSPHP (SEQ ID NO: 82), GSPHS (SEQ ID NO: 83), ASPHP (SEQ ID NO: 84), LSPHY (SEQ ID NO: 85), MSPHS (SEQ ID NO: 86), SSPHW (SEQ ID NO: 87), ASPHY (SEQ ID NO: 88), NSPHG (SEQ ID NO: 89), RSPHY (SEQ ID NO: 90), LSPHT (SEQ ID NO: 91), SSPHA (SEQ ID NO: 92), FSPHS (SEQ ID NO: 93), LSPHG (SEQ ID NO: 94), TSPHS (SEQ ID NO: 95), RSPHN (SEQ ID NO: 96), GSPHL (SEQ ID NO: 97), FSPHG (SEQ ID NO: 98), RSPHP (SEQ ID NO: 99), YSPHS (SEQ ID NO: 100), MSPHQ (SEQ ID NO: 101), RSPHG (SEQ ID NO: 102), RSPHS (SEQ ID NO: 103), GSPHQ (SEQ ID NO: 104), TSPHL (SEQ ID NO: 105), RSPHM (SEQ ID NO: 106), QSPHI (SEQ ID NO: 107), TSPHA (SEQ ID NO: 108), VSPHQ (SEQ ID NO: 109), LSPHA (SEQ ID NO: 110), RSPHA (SEQ ID NO: 111), RSPHT (SEQ ID NO: 112), LSPHS (SEQ ID NO: 113), SSPHS (SEQ ID NO: 114), TSPHV (SEQ ID NO: 115), QSPHG (SEQ ID NO: 116), KSPHW (SEQ ID NO: 117), NSPHH (SEQ ID NO: 118), LSPHV (SEQ ID NO: 119), ASPHN (SEQ

ID NO: 120), MSPHV (SEQ ID NO: 121), VSPHP (SEQ ID NO: 122), LSPHH (SEQ ID NO: 123), VSPHA (SEQ ID NO: 124), ISPHL (SEQ ID NO: 125), RSPHQ (SEQ ID NO: 126), YSPHT (SEQ ID NO: 127), LSPHL (SEQ ID NO: 128), ASPHS (SEQ ID NO: 129), HSPHG (SEQ ID NO: 130), KSPHS (SEQ ID NO: 131), TSPHT (SEQ ID NO: 132), or TSPHW (SEQ ID NO: 133).

31. The AAV capsid variant of any one of embodiments 1-30, wherein the amino acid sequence comprises any one of SEQ ID NOs: 1382, 1384, 1390, 1407, 1411, 1423, 1427, 1434, 1445, 1462, 1471, 1473, 1476, 1480, 1485-1487, 1492, 1493, 1495, 1496, 1500-1513, 1516-1519, 1522-1528, 1530-1539, 1541-1543, 1545, 1547, 1549, 1551-1555, or 1557-1566.

32. An AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1100-1571 or 1573-1735, optionally wherein the amino acid sequence is present in loop IV, e.g., immediately subsequent to position 453 or 455, numbered according to SEQ ID NO: 138 or 981.

33. The AAV capsid variant of any one of embodiments 21-32, wherein the amino acid sequence is present in loop IV, wherein loop IV is present at positions 449-460 numbered according to SEQ ID NO: 138.

34. The AAV capsid variant of embodiment 32 or 33, which comprises the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, 1379, 1382, 1384, 1387, 1390, 1391, 1396, 1406, 1407, 1409, 1410, 1411, 1423, 1427, 1431, 1434, 1440-1571, or 1573-1658.

35. The AAV capsid variant of embodiment 32 or 33, which comprises the amino acid sequence of any one of SEQ ID NOs: 1379, 1387, 1391, 1396, 1406, 1409, 1410, 1431, 1440-1444, 1446-1461, 1463-1470, 1472, 1474, 1475, 1477-1479, 1481-1484, 1488-1491, 1494, 1497-1499, 1514, 1515, 1520, 1521, 1529, 1540, 1544, 1546, 1548, 1550, or 1556.

36. The AAV capsid variant of embodiment 32 or 33, which comprises the amino acid sequence of any one of SEQ ID NOs: 1382, 1384, 1390, 1407, 1411, 1423, 1427, 1434, 1445, 1462, 1471, 1473, 1476, 1480, 1485-1487, 1492, 1493, 1495, 1496, 1500-1513, 1516-1519, 1522-1528, 1530-1539, 1541-1543, 1545, 1547, 1549, 1551-1555, or 1557-1566.

37. The AAV capsid variant of embodiment 32 or 33, which comprises the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1567, 1568, 1569, 1570, or 1571.

38. The AAV capsid variant of embodiment 32 or 33, which comprises the amino acid sequence of any one of SEQ ID NOs: 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, or 1573-1612.

39. The AAV capsid variant of embodiment 32 or 33, which comprises the amino acid sequence of any one of SEQ ID NOs: 1613-1658.

40. The AAV capsid variant of any one of embodiments 21-39, wherein the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138 or 982.

41. The AAV capsid variant of any one of embodiments 21-40, wherein the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 138 or 982.

42. The AAV capsid variant of any one of embodiments 21-31, 33, 40, or 41, wherein X_1 is present at position 454, X_2 is present at position 455, S is present at position 456, P is present at position 457, H is present at position 458, and X_3 is present at position 459, numbered according to SEQ ID NO: 138 or 982.

43. The AAV capsid variant of embodiment 32 or 33, which comprises the amino acid sequence of any one of SEQ ID NOs: 1100-1439.

44. The AAV capsid variant of embodiment 32 or 33, which comprises the amino acid sequence of any one of SEQ ID NOs: 1100-1283.

45. The AAV capsid variant of embodiment 32 or 33, which comprises the amino acid sequence of any one of SEQ ID NOs: 1284-1376.

46. The AAV capsid variant of embodiment 32 or 33, which comprises the amino acid sequence of any one of SEQ ID NOs: 1377-1437.

47. The AAV capsid variant of embodiment 32 or 33, which comprises the amino acid sequence of SEQ ID NO: 1438 or 1439.

48. The AAV capsid variant of any one of embodiments 32, 33, or 43-47, wherein the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138 or 981.

49. The AAV capsid variant of any one of embodiments 32, 33, or 43-48, wherein the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 981 or 138.

50. An AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, 1379, 1382, 1384, 1387, 1390, 1391, 1396, 1406, 1407, 1409, 1410, 1411, 1423, 1427, 1431, 1434, 1440-1571, 1573-1658, 1659, or 1660, wherein the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138 or 982.

51. An AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1379, 1387, 1391, 1396, 1406, 1409, 1410, 1431, 1440-1444, 1446-1461, 1463-1470, 1472, 1474, 1475, 1477-1479, 1481-1484, 1488-1491, 1494, 1497-1499, 1514, 1515, 1520, 1521, 1529, 1540, 1544, 1546, 1548, 1550, or 1556, wherein the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138 or 982.

52. The AAV capsid variant of embodiment 50 or 51, wherein the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 138 or 982.

53. An AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1100-1439 or 1661-1663, wherein the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138 or 981.

54. An AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1100-1283 or 1662-1663, wherein the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138 or 981.

55. The AAV capsid variant of embodiment 53 or 54, wherein the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 138 or 981.

56. The AAV capsid variant of any one of the preceding embodiments which is capable of binding to a glycosylphosphatidylinositol (GPI) anchored protein, e.g., alkaline phosphatase (ALPL).

57. The AAV capsid variant of any one of the preceding embodiments which demonstrates preferential transduction in a cell comprising a glycosylphosphatidylinositol (GPI) anchored protein, e.g., alkaline phosphatase (ALPL), relative to a cell that does not comprise a glycosylphosphatidylinositol (GPI) anchored protein, e.g., as measured by an assay, e.g., an assay of Example 4.

58. The AAV capsid variant of any one of the preceding embodiments which is enriched at least 292, 250, 230, 220, 215, 210, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 105, 100, 90, 95, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, 5, 4, 3, or 2-fold in a cell comprising a glycosylphosphatidylinositol (GPI) anchored protein, e.g., alkaline phosphatase (ALPL), e.g., relative to levels present prior to transduction, e.g., as measured by an assay, e.g., an assay of Example 4.

59. The AAV capsid variant of any one of embodiments 56-58, wherein the GPI anchored protein is conserved in at least two to three species, e.g., at least three species (e.g., mice, NHPs (e.g., *Macaca fascicularis*), and/or humans).

60. The AAV capsid variant of any one of embodiments of 56-59, wherein the GPI anchored protein is present on the surface of a cell in the blood brain barrier; and/or wherein the GPI anchored protein is ALPL.

61. The AAV capsid variant of any one of the preceding embodiments, which further comprises:

(i) a modification, e.g., an insertion, substitution (e.g., conservative substitution), and/or deletion, in loop I, II, VI and/or VIII; and/or

(ii) a substitution at position K449, e.g., a K449R substitution, numbered according to SEQ ID NO: 138.

62. The AAV capsid variant of any one of the preceding embodiments, which comprises a VP1 protein, a VP2 protein, a VP3 protein, or a combination thereof.

63. The AAV capsid variant of any one of embodiments 1-62, which further comprises the amino acid sequence corresponding to positions 138-736, e.g., a VP2, of SEQ ID NO: 138, or a sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

64. The AAV capsid variant of any one of embodiments 1-63, which further comprises the amino acid sequence corresponding to positions 203-736, e.g., a VP3, of SEQ ID NO: 138, or a sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

65. The AAV capsid variant of any one of the preceding embodiments, which further comprises an amino acid sequence comprising at least one, two or three modifications, e.g., substitutions (e.g., conservative substitutions), but not more than 30, 20 or 10 modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of SEQ ID NO: 138.

66. The AAV capsid variant of any one of the preceding embodiments, which further comprises an amino acid sequence comprising at least one, two or three, but no more than 30, 20 or 10 different amino acids relative to the amino acid sequence of SEQ ID NO: 138.

67. The AAV capsid variant of any one of the preceding embodiments, which further comprises the amino acid sequence of SEQ ID NO: 138, or an amino acid sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

68. The AAV capsid variant of any one of the preceding embodiments, which further comprises the amino acid sequence of SEQ ID NO: 138.

69. The AAV capsid variant of any one of the preceding embodiments, wherein:

(i) the AAV capsid variant further comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 137, or a sequence with at least 80% (e.g., at least about 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto; and/or

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

70. A polynucleotide encoding the AAV capsid variant of any one of embodiments 1-69.

71. The polynucleotide of embodiment 70, which comprises a nucleotide sequence that is codon optimized.

72. A peptide comprising the amino acid sequence of any one of SEQ ID NOs: 1100-1571 or 1573-1735.

73. The peptide of embodiment 72, which is fused or coupled, e.g., conjugated, to an active agent, e.g., a therapeutic agent or a diagnostic agent.

74. The peptide of embodiment 72 or 73, wherein at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides are fused or coupled, e.g., conjugated, to an active agent, e.g., a therapeutic agent or a diagnostic agent.

75. The peptide of embodiment 74, wherein the at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides comprise the same amino acid sequence.

76. The peptide of embodiment 74, wherein the at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides comprise different amino acid sequences.

77. The peptide of any one of embodiments 74-76, wherein the at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides are present in tandem (e.g., connected directly or indirectly via a linker) or in a multimeric configuration.

78. The peptide of any one of embodiments 72-77, wherein the peptide comprises an amino acid sequence of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 20, 25, 30, or 35 amino acids in length.

79. The peptide of any one of embodiments 73-78, wherein the active agent is or comprises a therapeutic agent chosen from a protein (e.g., an enzyme), an antibody molecule, a nucleic acid molecule (e.g., an RNAi agent), or a small molecule.

80. The peptide of any one of embodiments 73-78, wherein the active agent is or comprises a ribonucleic acid complex (e.g., a Cas9/gRNA complex), a plasmid, a closed-end DNA, a circ-RNA, or an mRNA.

81. The peptide of any one of embodiments 73-78, wherein the active agent is an RNAi agent.

82. The peptide of embodiment 81, wherein the RNAi agent is a dsRNA, a siRNA, a shRNA, a pre-miRNA, a pri-miRNA, a miRNA, a stRNA, a lncRNA, a piRNA, an antisense oligonucleotide agent (ASO), or a snoRNA, optionally wherein the RNAi agent is an siRNA or an ASO, which further optionally comprises at least one modified nucleotide.

83. The peptide of any one of embodiments 73-82, wherein the active agent modulates, e.g., inhibits, decreases or increases, expression of, a CNS related gene, mRNA, and/or protein.

84. The peptide of any one of embodiments 73-83, wherein the active agent is a diagnostic agent is or comprises an imaging agent (e.g., a protein or small molecule compound coupled to a detectable moiety).

85. The peptide of any one of embodiments 73-84, wherein the peptide covalently linked, e.g., directly or indirectly via a linker, to the active agent.

86. The peptide of any one of embodiments 73-85, wherein the peptide is conjugated to the active agent via a linker.

87. The peptide of embodiment 86, wherein the linker is a cleavable linker or a non-cleavable linker.

88. The peptide of embodiment 87, wherein the cleavable linker is a pH sensitive linker or an enzyme sensitive linker.

89. The peptide of embodiment 87 or 88, wherein:

(i) the pH sensitive linker comprises a hydrazine/hydrazone linker or a disulfide linker;

(ii) the enzyme sensitive linker comprises a peptide based linker, e.g., a peptide linker sensitive to a protease (e.g., a lysosomal protease); or a beta-glucuronide linker; or

(iii) the non-cleavable linker is a linker comprising a thioether group or a maleimidocaproyl group.

90. The peptide of any one of embodiments 73-89, wherein:

(i) the peptide and the active agent are fused or coupled post-translationally, e.g., using click chemistry; or

(ii) the peptide and the active agent are fused or couple via chemically induced dimerization.

91. The peptide of any one of embodiments 73-90, wherein the peptide is present N-terminal relative to the active agent.

92. The peptide of any one of embodiments 73-90, wherein the peptide is present C-terminal relative to the active agent.

93. The peptide of any one of embodiment 73-78, 83, or 85-92, wherein the peptide is present or coupled to a carrier, e.g., an exosome, a microvesicle, or a lipid nanoparticle (LNP), optionally, wherein the carrier comprises a therapeutic agent (e.g., an RNAi agent (e.g., an dsRNA, a siRNA, a shRNA, a pre-miRNA, a pri-miRNA, a miRNA, a stRNA, a lncRNA, a piRNA, an antisense oligonucleotide agent (ASO), or a snoRNA), an mRNA, a ribonucleoprotein complex (e.g., a Cas9/gRNA complex), or a circRNA).

94. The peptide of embodiment 93, wherein the peptide is present on the surface of the carrier, optionally wherein at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the surface of the carrier comprises at least 1-5, e.g., at least 1, 2, 3, 4, or 5 peptides according to any one of embodiments 422-436.

95. A polynucleotide encoding an AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1100-1571 or 1573-1658.

96. The polynucleotide, peptide, or AAV capsid variant, of any one of embodiments 1-95, which is isolated, e.g., recombinant.

97. An AAV particle comprising the AAV capsid variant of any one of embodiments 1-69, or 96.

98. The AAV particle of embodiment 97, which comprises a nucleotide sequence encoding a payload.

99. The AAV particle of embodiment 98, wherein the encoded payload comprises a therapeutic protein or functional variant thereof; an antibody or antibody fragment; an enzyme; a component of a gene editing system; an RNAi agent (e.g., a dsRNA, siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA); or a combination thereof.

100. The AAV particle of embodiment 99, wherein the therapeutic protein or functional variant thereof, e.g., a recombinant protein, is associated with (e.g., aberrantly expressed in) a neurological or neurodegenerative disorder, a muscular or neuromuscular disorder, or a neuro-oncological disorder.

101. The AAV particle of embodiment 99 or 100, the therapeutic protein or functional variant thereof is chosen from apolipoprotein E (APOE) (e.g., ApoE2, ApoE3 and/or ApoE4); human survival of motor neuron (SMN) 1 or SMN2; aromatic L-amino acid decarboxylase (AADC); aspartoacylase (ASPA); tripeptidyl peptidase I (CLN2); beta-galactosidase (GLB1); N-sulphoglucosamine sulphohydrolase (SGSH); N-acetyl-alpha-glucosaminidase (NAGLU); iduronate 2-sulfatase (IDS); intracellular cholesterol transporter (NPC1); gigaxonin (GAN); or a combination thereof.

102. The AAV particle of embodiment 99, wherein the antibody or antibody binding fragment binds to:

(i) a CNS related target, e.g., an antigen associated with a neurological or neurodegenerative disorder, e.g., β-amyloid, APOE, tau, SOD1, TDP-43, huntingtin (HTT), and/or synuclein;

(ii) a muscular or neuromuscular related target, e.g., an antigen associated with a muscular or neuromuscular disorder; or

(iii) a neuro-oncology related target, e.g., an antigen associated with a neuro-oncological disorder, e.g., HER2, or EGFR (e.g., EGFRvIII).

103. The AAV particle of embodiment 99, wherein the enzyme comprises a meganuclease, a zinc finger nuclease, a TALEN, a recombinase, integrase, a base editor, a Cas9, or a fragment thereof.

104. The AAV particle of embodiment 99, wherein the component of a gene editing system comprises one or more components of a CRISPR-Cas system.

105. The AAV particle of embodiment 104, wherein the one or more components of the CRISPR-Cas system comprises a Cas9, e.g., a Cas9 ortholog or a Cpf1, and a single guide RNA (sgRNA), optionally wherein:

(i) the sgRNA is located upstream (5') of the cas9 enzyme; or

(ii) the sgRNA is located downstream (3') of the cas9 enzyme.

106. The AAV particle of embodiment 99, wherein the RNAi agent (e.g., a dsRNA, siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA), modulates, e.g., inhibits, expression of, a CNS related gene, mRNA, and/or protein.

107. The AAV particle of embodiment 106, wherein the CNS related gene is chosen from SOD1, MAPT, APOE, HTT, TDP-43, APP, BACE, SNCA, ATXN1, ATXN3, ATXN7, SCN1A-SCN5A, SCN8A-SCN11A, or a combination thereof.

108. The AAV particle of any one of embodiments 97-107, which comprises a viral genome comprising a promoter operably linked to the nucleic acid sequence encoding the payload.

109. The AAV particle of embodiment 108, wherein the promoter is chosen from human elongation factor 1α-subunit (EF1α), cytomegalovirus (CMV) immediate-early enhancer and/or promoter, chicken β-actin (CBA) and its derivative CAG, β glucuronidase (GUSB), or ubiquitin C (UBC), neuron-specific enolase (NSE), platelet-derived growth factor (PDGF), platelet-derived growth factor B-chain (PDGF-β), intercellular adhesion molecule 2 (ICAM-2), synapsin (Syn), methyl-CpG binding protein 2 (MeCP2), Ca2+/calmodulin-dependent protein kinase II (CaMKII), metabotropic glutamate receptor 2 (mGluR2), neurofilament light (NFL) or heavy (NFH), β-globin minigene nβ2, preproenkephalin (PPE), enkephalin (Enk) and excitatory amino acid transporter 2 (EAAT2), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), 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 fragment, e.g., a truncation, or a functional variant thereof.

110. The AAV particle of embodiment 108 or 109, wherein the promoter is an EF-1a promoter variant, e.g., a truncated EF-1a promoter.

111. The AAV particle of any one of embodiments 108-110, wherein the promoter comprises the nucleotide sequence of any one of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998-1007 or any one of the sequences provided in Table 8, a nucleotide sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998-1007 or any one of the

sequences provided in Table 8, or a nucleotide sequence with at least 80% (e.g., 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998-1007 or any one of the sequences provided in Table 8.

112. The AAV particle of any one of embodiments 108-111 wherein the viral genome further comprises a polyA signal sequence.

113. The AAV particle of any one of embodiments 108-112, wherein the viral genome further comprises an inverted terminal repeat (ITR) sequence.

114. The AAV particle of any one of embodiments 108-113, wherein the viral genome comprises an ITR sequence positioned 5' relative to the encoded payload.

115. The AAV particle of any one of embodiments 108-114, wherein the viral genome comprises an ITR sequence positioned 3' relative to the encoded payload.

116. The AAV particle of any one of embodiments 108-115, wherein the viral genome comprises an ITR sequence positioned 5' relative to the encoded payload and an ITR sequence positioned 3' relative to the encoded payload.

117. The AAV particle of any one of embodiments 108-116, wherein the viral genome further comprises an enhancer, a Kozak sequence, an intron region, and/or an exon region.

118. The AAV particle of any one of embodiments 108-117, wherein the viral genome further comprises a nucleotide sequence encoding a miR binding site, e.g., a miR binding site that modulates, e.g., reduces, expression of the antibody molecule encoded by the viral genome in a cell or tissue where the corresponding miRNA is expressed.

119. The AAV particle of embodiment 118, wherein the encoded miRNA binding site is complementary, e.g., fully complementary or partially complementary, to a miRNA expressed in a cell or tissue of the DRG, liver, heart, hematopoietic, or a combination thereof.

120. The AAV particle of embodiment 118 or 119, wherein the encoded miR binding site modulates, e.g., reduces, expression of the encoded antibody molecule in a cell or tissue of the DRG, liver, heart, hematopoietic lineage, or a combination thereof.

121. The AAV particle of any one of embodiments 108-120, wherein the viral genome comprises at least 1-5 copies of the encoded miR binding site, e.g., at least 1, 2, 3, 4, or 5 copies.

122. The AAV particle of any one of embodiments 108-121, wherein the viral genome comprises at least 3 copies of an encoded miR binding sites, optionally wherein all three copies comprise the same miR binding site, or at least one, two, three, or all of the copies comprise a different miR binding site.

123. The AAV particle of embodiment 122, wherein the 3 copies of the encoded miR binding sites are continuous (e.g., not separated by a spacer), or are separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of GATAGTTA.

124. The AAV particle of any one of embodiments 108-123, wherein the viral genome comprises at least 4 copies of an encoded miR binding site, optionally wherein all four copies comprise the same miR binding site, or at least one, two, three, or all of the copies comprise a different miR binding site.

125. The AAV particle of embodiment 124, wherein the 4 copies of the encoded miR binding sites are continuous (e.g., not separated by a spacer), or are separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of GATAGTTA.

126. The AAV particle of any one of embodiments 118-125, wherein the encoded miR binding site comprises a miR122 binding site, a miR183 binding site, a miR-1 binding site, a miR-142-3p, or a combination thereof, optionally wherein:

(i) the encoded miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4673;

(ii) the encoded miR183 binding site comprises the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at

least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4676;

(iii) the encoded miR-1 binding site comprises the nucleotide sequence of SEQ ID NO: 4679, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4679; and/or

(iv) the encoded miR-142-3p binding site comprises the nucleotide sequence of SEQ ID NO: 4675, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4675.

127. The AAV particle of any one of embodiments 108-126, wherein the viral genome comprises an encoded miR122 binding site.

128. The AAV particle of any one of embodiments 108-127, wherein the viral genome comprises at least 1-5 copies, e.g., 1, 2, or 3 copies of a miR122 binding site, optionally wherein each copy is continuous (e.g., not separated by a spacer), or each copy is separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of GATAGTTA.

129. The AAV particle of embodiment 127 or 128, wherein the encoded miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4673.

130. The AAV particle of any one of embodiments 108-129, wherein the viral genome comprises:

(A) (i) a first encoded miR122 binding site comprising the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%,

90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4673;

(ii) a first spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of GATAGTTA; and

(iii) a second encoded miR122 binding site comprising the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4673; or

(B) (i) a first encoded miR122 binding site comprising the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4673;

(ii) a first spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of GATAGTTA;

(iii) a second encoded miR122 binding site comprising the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4673;

(iv) a second spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of GATAGTTA; and

(v) a third encoded miR122 binding site comprising the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at

least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4673.

131. The AAV particle of any one of embodiments 108-130, wherein the viral genome comprises an encoded miR183 binding site.

132. The AAV particle of any one of embodiments 108-131, wherein the viral genome comprises at least 1-5 copies, e.g., 1, 2, or 3 copies of a miR183 binding site, optionally wherein each copy is continuous (e.g., not separated by a spacer), or each copy is separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of GATAGTTA.

133. The AAV particle of embodiment 131 or 132, wherein the encoded miR183 binding site comprises the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4673.

134. The AAV particle of any one of embodiments 108-133, wherein the viral genome comprises:
(A) (i) a first encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4676;

(ii) a first spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of GATAGTTA; and

(iii) a second encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative

substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4676; or

(B) (i) a first encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4676;

(ii) a first spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to GATAGTTA;

(iii) a second encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4676;

(iv) a second spacer comprising the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of GATAGTTA; and

(v) a third encoded miR183 binding site comprising the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4676.

135. The AAV particle of any one of embodiments 108-134, wherein the viral genome comprises an encoded miR122 binding site and a miR-1 binding site.

136. The AAV particle of any one of embodiments 108-135, wherein the viral genome is single stranded.

137. The AAV particle of any one of embodiments 108-135, wherein the viral genome self-complementary.

138. The AAV particle of any one of embodiments 108-137, wherein the viral genome further comprises a nucleotide sequence encoding a Rep protein, e.g., a non-structural protein, wherein the Rep protein comprises a Rep78 protein, a Rep68, Rep52 protein, and/or a Rep40 protein (e.g., a Rep78 and a Rep52 protein).

139. The AAV particle of any one of embodiments 108-137, wherein the AAV particle further comprises a nucleotide sequence encoding a Rep protein, e.g., a non-structural protein, wherein the Rep protein comprises a Rep78 protein, a Rep68, Rep52 protein, and/or a Rep40 protein (e.g., a Rep78 and a Rep52 protein).

140. The AAV particle of embodiment 138 or 139, wherein the Rep78 protein, the Rep68 protein, the Rep52 protein, and/or the Rep40 protein are encoded by at least one Rep gene.

141. The AAV particle of any one of embodiments 108-140, wherein the viral genome further comprises a nucleic acid sequence encoding the AAV capsid variant of any one of embodiments 1-519, 566, or 574.

142. The AAV particle of any one of embodiments 97-141, which is isolated, e.g., recombinant.

143. A vector comprising a polynucleotide encoding the AAV capsid variant of any one of embodiments 1-69, or 96, the polynucleotide of any one of embodiments 70, 71, 95, or 96, or a polynucleotide encoding the peptide of embodiment 72 or 96.

144. A cell, e.g., a host cell, comprising the AAV capsid variant of any one of embodiments 1-69, or 96, the polynucleotide of any one of embodiments 70, 71, 95, or 96, the peptide of any one of embodiments 72-94 or 96, the AAV particle of any one of embodiments 97-142, or the vector of embodiment 143.

145. The cell of embodiment 144, wherein the cell is a mammalian cell or an insect cell.

146. The cell of embodiment 144 or 145, wherein the cell is a cell of a brain region or a spinal cord region, optionally a cell of the brain stem, hippocampus, or thalamus.

147. The cell of any one of embodiments 144-146, wherein the cell is a neuron, a sensory neuron, a motor neuron, an astrocyte, a glial cell, or oligodendrocyte.

148. A method of making an AAV particle, comprising

(i) providing a host cell comprising a viral genome; and

(ii) incubating the host cell under conditions suitable to enclose the viral genome in the AAV capsid variant of any one of embodiments 1-69, or 96 or an AAV capsid variant encoded by the polynucleotide of any one of embodiments 70, 71, 95, or 96;

thereby making the AAV particle.

149. The method of embodiment 148, further comprising, prior to step (i), introducing a first nucleic acid molecule comprising the viral genome into the host cell.

150. The method of embodiment 148 or 149, wherein the host cell comprises a second nucleic acid encoding the capsid variant.

151. The method of embodiment 150, wherein the second nucleic acid molecule is introduced into the host cell prior to, concurrently with, or after the first nucleic acid molecule.

152. A pharmaceutical composition comprising the AAV particle of any one of embodiments 97-142, an AAV particle comprising the capsid variant of any one of embodiments 1-69, or 96, an AAV particle comprising the peptide of embodiment 72 or 96, and a pharmaceutically acceptable excipient.

153. A method of delivering a payload to a cell or tissue (e.g., a CNS cell, CNS tissue, a liver cell, or a liver tissue), comprising administering an effective amount of the pharmaceutical composition of embodiment 152, the AAV particle of any one of embodiments 97-142, an AAV particle comprising the capsid variant of any one of embodiments 1-69 or 96, or an AAV particle comprising the peptide of embodiment 69 or 96.

154. The method of embodiment 153, wherein the cell is a cell of a brain region or a spinal cord region, optionally a cell of the frontal cortex, sensory cortex, motor cortex, caudate, cerebellar cortex, cerebral cortex, brain stem, hippocampus, or thalamus.

155. The method of embodiment 153 or 154, wherein the cell is a neuron, a sensory neuron, a motor neuron, an astrocyte, a glial cell, or an oligodendrocyte.

156. The method of any one of embodiments 153-154, wherein the cell or tissue is within a subject.

157. The method of embodiment 156, wherein the subject has, has been diagnosed with having, or is at risk of having a genetic disorder, e.g., a monogenic disorder or a polygenic disorder.

158. The method of embodiment 156 or 157, wherein the subject has, has been diagnosed with having, or is at risk of having a neurological, e.g., a neurodegenerative disorder.

159. The method of embodiment 156 or 157, wherein the subject has, has been diagnosed with having, or is at risk of having a neuro-oncological disorder.

160. The method of embodiment 156 or 157, wherein the subject has, has been diagnosed with having, or is at risk of having a muscular disorder or a neuromuscular disorder.

161. A method of treating a subject having or diagnosed with having a genetic disorder, e.g., a monogenic disorder or a polygenic disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of embodiment 152, the AAV particle of any one of embodiments 97-142, an AAV particle comprising the capsid variant of any one of embodiments 1-69 or 96, or an AAV particle comprising the peptide of embodiment 69 or 96.

162. A method of treating a subject having or diagnosed with having a neurological disorder, e.g., a neurodegenerative disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of embodiment 152, the AAV particle of any one of embodiments 97-142, an AAV particle comprising the capsid variant of any one of embodiments 1-69 or 96, or an AAV particle comprising the peptide of embodiment 69 or 96.

163. A method of treating a subject having or diagnosed with having a muscular disorder or a neuromuscular disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of embodiment 152, the AAV particle of any one of embodiments 97-142, an AAV particle comprising the capsid variant of any one of embodiments 1-69 or 96, or an AAV particle comprising the peptide of embodiment 69 or 96.

164. A method of treating a subject having or diagnosed with having a neuro-oncological disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of embodiment 152, the AAV particle of any one of embodiments 97-142, an AAV particle comprising the capsid variant of any one of embodiments 1-69 or 96, or an AAV particle comprising the peptide of embodiment 69 or 96.

165. The method of any one of embodiments 157-164, wherein the genetic disorder, neurological disorder, neurodegenerative disorder, muscular disorder, neuromuscular disorder, or neuro-oncological disorder is Huntington's Disease, Amyotrophic Lateral Sclerosis (ALS), Gaucher Disease, Dementia with Lewy Bodies, Parkinson's disease, Spinal Muscular Atrophy, Alzheimer's

Disease, a leukodystrophy (e.g., Alexander disease, autosomal dominant leukodystrophy with autonomic diseases (ADLD), Canavan disease, cerebrotendinous xanthomatosis (CTX), metachromatic leukodystrophy (MLD), Pelizaeus-Merzbacher disease, or Refsum disease), or a cancer (e.g., a HER2/neu positive cancer or a glioblastoma).

166. The method of any one of embodiments 161-165, where treating comprises prevention of progression of the disease or disorder in the subject.

167. The method of embodiment 156-166, wherein the subject is a human.

168. The method of any one of embodiments 156-167, wherein the AAV particle is administered to the subject intravenously, via intra-cisterna magna injection (ICM), intracerebrally, intrathecally, intracerebroventricularly, via intraparenchymal administration, intraarterially, or intramuscularly.

169. The method of any one of embodiments 156-168, wherein the AAV particle is administered to the subject via focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration.

170. The method of any one of embodiments 156-169, wherein the AAV particle is administered to the subject intravenously.

171. The method of any one of embodiments 156-170, wherein the AAV particle is administered to the subject via intra-cisterna magna injection (ICM).

172. The method of any one of embodiments 156-171, wherein the AAV particle is administered to the subject intraarterially.

173. The method of any one of embodiments 161-172, wherein administration of the AAV particle results in a decreased presence, level, and/or activity of a gene, mRNA, protein, or combination thereof.

174. The method of any one of embodiments 161-172, wherein administration of the AAV particle results in an increased presence, level, and/or activity of a gene, mRNA, protein, or a combination thereof.

173. The pharmaceutical composition of embodiment 152, the AAV particle of any one of embodiments 97-142, an AAV particle comprising the capsid variant of any one of embodiments 1-69

or 96, or an AAV particle comprising the peptide of embodiment 69 or 96, for use in a method of delivering a payload to a cell or tissue.

174. The pharmaceutical composition of embodiment 152, the AAV particle of any one of embodiments 97-142, an AAV particle comprising the capsid variant of any one of embodiments 1-69 or 96, or an AAV particle comprising the peptide of embodiment 69 or 96, for use in a method of treating a genetic disorder, a neurological disorder, a neurodegenerative disorder, a muscular disorder, a neuromuscular disorder, or a neuro-oncological disorder.

175. The pharmaceutical composition of embodiment 152, the AAV particle of any one of embodiments 97-142, an AAV particle comprising the capsid variant of any one of embodiments 1-69 or 96, or an AAV particle comprising the peptide of embodiment 69 or 96, for use in the manufacture of a medicament.

176. Use of the pharmaceutical composition of embodiment 152, the AAV particle of any one of embodiments 97-142, an AAV particle comprising the capsid variant of any one of embodiments 1-69 or 96, or an AAV particle comprising the peptide of embodiment 69 or 96, in the manufacture of a medicament.

177. Use of the pharmaceutical composition of embodiment 152, the AAV particle of any one of embodiments 97-142, an AAV particle comprising the capsid variant of any one of embodiments 1-69 or 96, or an AAV particle comprising the peptide of embodiment 69 or 96, in the manufacture of a medicament for treating a genetic disorder, a neurological disorder, a neurodegenerative disorder, a muscular disorder, a neuromuscular disorder, or a neuro-oncological disorder.

[0024] The details of one or more embodiments of the disclosure are set forth in the accompanying description below. Other features, objects and advantages of the disclosure will be apparent from the description. In the description, the singular forms also include the plural unless the context clearly dictates otherwise. Certain terms are defined in the Definition section and throughout.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] **FIG. 1A** is a violin plot showing expression level of the payload on the Y-axis in various cell types as shown on the X-axis, which includes from left to right, microglia, astrocytes, endothelial cells subset 1, vascular smooth cells, pericytes, endothelial cells subset 2, committed oligodendrocytes, macrophages, vascular and leptomeningeal cells, committed oligodendrocytes subset 2, and mature oligodendrocytes. **FIG. 1B** is a violin plot showing expression level of ALPL on the Y-axis in various cell types as shown on the X-axis, which includes from left to right, microglia,

astrocytes, endothelial cells subset 1, vascular smooth cells, pericytes, endothelial cells subset 2, committed oligodendrocytes, macrophages, vascular and leptomeningeal cells, committed oligodendrocytes subset 2, and mature oligodendrocytes.

[0026] **FIG. 2A** and **FIG. 2C** are graphs showing TTM-002 binding to ALPL at increasing concentrations of AAV by surface plasmon resonance (SPR) over time. **FIG. 2B** and **FIG. 2D** are graphs showing AAV9 binding to ALPL at increasing concentrations of AAV by SPR over time.

[0027] **FIG. 3** is a graph showing the luciferase activity (RLU) as a measure of TTM-002 (right side of graph) or AAV9 (left side of graph) at 24-hours post-transduction and 48-hours post-transfection with siRNA 1, 2 or both siRNA 1 and 2 targeting ALPL or a non-ALPL control siRNA that did not knockdown ALPL.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0028] Described herein, *inter alia*, are compositions comprising an AAV capsid variant, e.g., an AAV capsid variant described herein, and methods of making and using the same. Generally, the AAV capsid variant has enhanced tropism for a cell or tissue, e.g., for the delivery of a payload to said cell or tissue, for example a CNS tissue or a CNS cell or a liver cell or liver tissue.

[0029] As demonstrated in the Examples herein below, certain AAV capsid variants described herein show multiple advantages over wild-type AAV9, including (i) increased penetrance through the blood brain barrier following intravenous administration, (ii) wider distribution throughout the multiple brain regions, e.g., frontal cortex, sensory cortex, motor cortex, putamen, thalamus, cerebellar cortex, dentate nucleus, caudate, and/or hippocampus, and/or (iii) elevated payload expression in multiple brain regions. Without wishing to be being bound by theory, it is believed that these advantages may be due, in part, to the dissemination of the AAV capsid variants through the brain vasculature. In some embodiments, the AAV capsids described herein enhance the delivery of a payload to multiple regions of the brain including for example, the frontal cortex, sensory cortex, motor cortex, putamen, thalamus, cerebellar cortex, dentate nucleus, caudate, and/or hippocampus.

[0030] Several approaches have been used previously to produce AAV capsids with enhanced tropism for a cell or tissue, e.g., a CNS cell or tissue. One approach used co-infection of cultured cells (Grimm et al. In vitro and in vivo gene therapy vector evolution via multispecies interbreeding and retargeting of adeno-associated viruses. J. Virol. 2008 June 82(12):5887–5911, the contents of which are herein incorporated by reference in its entirety) or *in situ* animal tissue (Lisowski et al. Selection and evaluation of clinically relevant AAV variants in a xenograft liver model. Nature 2014 506:382-386, the contents of which are herein incorporated by reference in its entirety) with adenovirus, in order to trigger exponential replication of infectious AAV DNA. Another approach involved the use of cell-specific CRE transgenic mice (Deverman et al. Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain. Nat Biotechnol. 2016 Feb. 34(2)204-209; the contents of which are herein incorporated by reference in its entirety) allowing viral DNA recombination

specifically in astrocytes, followed by recovery of CRE-recombined capsid variants. Other approaches apply high throughput DNA synthesis, multiplexing, sequencing technologies, and machine learning to evaluate sequencing reads of viral DNA in different tissues to engineer variant capsids. These approaches are different from the approach disclosed herein.

[0031] There are some limitations to the art-known capsid generation methods. For example, the transgenic CRE system used by Deverman et al. (2016) has limited tractable in other animal species and AAV variants selected by directed evolution in mouse tissue do not show similar properties in large animals. Previously described transduction-specific approaches are not amenable to large animal studies because: 1) many tissues of interest (e.g., CNS) are not readily accessible to adenovirus co-infection, 2) the specific adenovirus tropism itself would bias the library distribution, and 3) large animals are typically not amenable to transgenesis or genetic engineering to express CRE recombinase in defined cell types.

[0032] To address these limitations, a broadly-applicable functional AAV capsid library screening platform for cell type-specific biopanning in non-transgenic animals has been developed and is described in the appended Examples. In the TRACER (Tropism Redirection of AAV by Cell type-specific Expression of RNA) platform system, the capsid gene is placed under the control of a cell type-specific promoter to drive capsid mRNA expression in the absence of helper virus co-infection. Without wishing to be bound by theory, it is believed that this RNA-driven screen increases the selective pressure in favor of capsid variants which transduce a specific cell type. The TRACER platform allows for generation of AAV capsid libraries whereby specific recovery and subcloning of capsid mRNA expressed in transduced cells is achieved with no need for transgenic animals or helper virus co-infection. Without wishing to be bound by theory, it is believed that since mRNA transcription is a hallmark of full transduction, the methods disclosed herein allow identification of fully infectious AAV capsid mutants, and in addition to its higher stringency, this method allows identification of capsids with high tropism for particular cell types using libraries designed to express CAP mRNA under the control of any cell-specific promoter such as, but not limited to, synapsin-1 promoter (neurons), GFAP promoter (astrocytes), TBG promoter (liver), CAMK promoter (skeletal muscle), MYH6 promoter (cardiomyocytes). Described herein are novel AAV capsid variants generated using the TRACER method which demonstrate enhance tropism in for example a CNS cell, a CNS tissue, a liver cell, a liver tissue, a muscle cell, or a muscle tissue.

[0033] In some embodiments, an AAV capsid variant disclosed herein comprises a modification in loop IV of AAV9, e.g., at positions between 449-460, e.g., at position 454 and/or 456, numbered relative to SEQ ID NO: 138, 981, or 982. In some embodiments, loop (e.g., loop IV) is used interchangeably herein with the term variable region (e.g., variable region IV), or VR (e.g., VR-IV). In some embodiments loop IV comprises positions 449-475 (e.g., amino acids KTINGSGQNQQTLKFSVAGPSNMAVQG (SEQ ID NO: 6404)), numbered according to SEQ ID

NO: 138. In some embodiments loop IV comprises positions 449-460 (e.g., amino acids KTINGSGQNQQT (SEQ ID NO: 6405)), numbered according to SEQ ID NO: 138. In some embodiments, loop IV or variable region IV (VR-IV) is as described in DiMattia et al. "Structural Insights into the Unique Properties of the Adeno-Associated Virus Serotype 9," *Journal of Virology*, 12(86):6947-6958 (the contents of which are hereby incorporated by reference in their entirety), e.g., comprising positions 452-460 (e.g., NGSGQNQQT (SEQ ID NO: 4487)), numbered according to SEQ ID NO: 138.

[0034] The AAV particles and payloads of the disclosure may be delivered to one or more target cells, tissues, organs, or organisms. In some embodiments, the AAV particles of the disclosure demonstrate enhanced tropism for a target cell type, tissue or organ. As a non-limiting example, the AAV particle may have enhanced tropism for cells and tissues of the central or peripheral nervous systems (CNS and PNS, respectively). In some embodiments, an AAV particle of the disclosure may, in addition, or alternatively, have decreased tropism for a cell-type, tissue or organ.

[0035] In some embodiments, an AAV comprises a small non-enveloped icosahedral capsid virus of the Parvoviridae family and is characterized by a single stranded DNA viral genome. Parvoviridae family viruses consist of two subfamilies: Parvovirinae, which infect vertebrates, and Densovirinae, which infect invertebrates. The Parvoviridae family comprises the Dependovirus genus which includes AAV, capable of replication in vertebrate hosts including, but not limited to, human, primate, bovine, canine, equine, and ovine species.

[0036] The parvoviruses and other members of the Parvoviridae family are generally described in Kenneth I. Berns, "Parvoviridae: The Viruses and Their Replication," Chapter 69 in FIELDS VIROLOGY (3d Ed. 1996), the contents of which are incorporated by reference in their entirety.

[0037] In some embodiments, AAV are used as a biological tool due to a relatively simple structure, their ability to infect a wide range of cells (including quiescent and dividing cells) without integration into the host genome and without replicating, and their relatively benign immunogenic profile. The genome of the virus may be manipulated to contain a minimum of components for the assembly of a functional recombinant virus, or viral particle, which is loaded with or engineered to target a particular tissue and express or deliver a desired payload.

[0038] In some embodiments, the AAV, is a naturally occurring (e.g., wild-type) AAV or a recombinant AAV. In some embodiments, the wild-type AAV vector genome is a linear, single-stranded DNA (ssDNA) molecule approximately 5,000 nucleotides (nt) in length. In some embodiments, inverted terminal repeats (ITRs) cap the viral genome at both the 5' and the 3' end, providing origins of replication for the viral genome. In some embodiments, an AAV viral genome typically comprises two ITR sequences. These ITRs have a characteristic T-shaped hairpin structure defined by a self-complementary region (145nt in wild-type AAV) at the 5' and 3' ends of the ssDNA which form an energetically stable double stranded region. The double stranded hairpin structures

comprise multiple functions including, but not limited to, acting as an origin for DNA replication by functioning as primers for the endogenous DNA polymerase complex of the host viral replication cell.

[0039] In some embodiments, the wild-type AAV viral genome further comprises nucleotide sequences for two open reading frames, one for the four non-structural Rep proteins (Rep78, Rep68, Rep52, Rep40, encoded by Rep genes) and one for the three capsid, or structural, proteins (VP1, VP2, VP3, encoded by capsid genes or Cap genes). The Rep proteins are used for replication and packaging, while the capsid proteins are assembled to create the protein shell of the AAV, or AAV capsid polypeptide, e.g., an AAV capsid variant. Alternative splicing and alternate initiation codons and promoters result in the generation of four different Rep proteins from a single open reading frame and the generation of three capsid proteins from a single open reading frame. Though it varies by AAV serotype, as a non-limiting example, for AAV9/hu.14 (SEQ ID NO: 123 of US 7,906,111, the contents of which are herein incorporated by reference in their entirety) VP1 refers to amino acids 1-736, VP2 refers to amino acids 138-736, and VP3 refers to amino acids 203-736. In some embodiments, for any one of the amino acid sequences of SEQ ID NO: 981 or 982, VP1 comprises amino acids 1-742, VP2 comprises amino acids 138-742, and VP3 comprises amino acids 203-742. In other words, VP1 is the full-length capsid sequence, while VP2 and VP3 are shorter components of the whole. As a result, changes in the sequence in the VP3 region, are also changes to VP1 and VP2, however, the percent difference as compared to the parent sequence will be greatest for VP3 since it is the shortest sequence of the three. Though described here in relation to the amino acid sequence, the nucleic acid sequence encoding these proteins can be similarly described. Together, the three capsid proteins assemble to create the AAV capsid protein. While not wishing to be bound by theory, the AAV capsid protein typically comprises a molar ratio of 1:1:10 of VP1:VP2:VP3.

[0040] AAV vectors of the present disclosure may be produced recombinantly and may be based on adeno-associated virus (AAV) reference sequences. In addition to single stranded AAV viral genomes (e.g., ssAAVs), the present disclosure also provides for self-complementary AAV (scAAVs) viral genomes. scAAV vector genomes contain DNA strands which anneal together to form double stranded DNA. By skipping second strand synthesis, scAAVs allow for rapid expression in the transduced cell. In some embodiments, the AAV particle of the present disclosure is an scAAV. In some embodiments, the AAV particle of the present disclosure is an ssAAV.

[0041] Methods for producing and/or modifying AAV particles are disclosed in the art such as pseudotyped AAV vectors (PCT Patent Publication Nos. WO200028004; WO200123001; WO2004112727; WO2005005610; and WO2005072364, the content of each of which is incorporated herein by reference in its entirety).

[0042] As described herein, the AAV particles of the disclosure comprising an AAV capsid variant, and a viral genome, have enhanced tropism for a cell-type or a tissue, e.g., a CNS cell-type, region, or tissue.

Peptides

[0043] Disclosed herein are peptides, and associated AAV particles comprising an AAV capsid variant and a peptide for enhanced or improved transduction of a target tissue (e.g., cells of the CNS or PNS). In some, embodiments, the peptide is an isolated, e.g., recombinant, peptide. In some embodiments, the nucleic acid encoding the peptide, is an isolated, e.g., recombinant nucleic acid.

[0044] In some embodiments, the peptide may increase distribution of an AAV particle to a cell, region, or tissue of the CNS. The cell of the CNS may be, but is not limited to, neurons (e.g., excitatory, inhibitory, motor, sensory, autonomic, sympathetic, parasympathetic, Purkinje, Betz, etc.), glial cells (e.g., microglia, astrocytes, oligodendrocytes) and/or supporting cells of the brain such as immune cells (e.g., T cells). The tissue of the CNS may be, but is not limited to, the cortex (e.g., frontal, parietal, occipital, and/or temporal), thalamus, hypothalamus, striatum, putamen, caudate nucleus, hippocampus, entorhinal cortex, basal ganglia, or deep cerebellar nuclei.

[0045] In some embodiments, the peptide may increase distribution of an AAV particle to a cell, region, or tissue of the PNS. The cell or tissue of the PNS may be, but is not limited to, a dorsal root ganglion (DRG).

[0046] In some embodiments, the peptide may increase distribution of an AAV particle to the CNS (e.g., the cortex) after intravenous administration. In some embodiments, the peptide may increase distribution of an AAV particle to the CNS (e.g., the cortex) following focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration.

[0047] In some embodiments, the peptide may increase distribution of an AAV particle to the PNS (e.g., DRG) after intravenous administration. In some embodiments, the peptide may increase distribution of an AAV particle to the PNS (e.g., DRG) following focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration.

[0048] In some embodiments, the peptide may increase distribution of an AAV particle to a cell, region, or tissue of a muscle. In some embodiments, the muscle is a heart muscle, e.g., a heart atrium or a heart ventricle. In some embodiments, the peptide may direct an AAV particle to a muscle cell, region, or tissue after intravenous administration.

[0049] In some embodiments, the peptide may increase distribution of an AAV particle to a cell, region, or tissue of the liver.

[0050] A peptide may vary in length. In some embodiments, the peptide is about 3 to about 20 amino acids in length. As non-limiting examples, the peptide may be 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 3-5, 3-8, 3-10, 3-12, 3-15, 3-18, 3-20, 5-10, 5-15, 5-20, 10-12, 10-15, 10-20, 12-20, or 15-20 amino acids in length. In some embodiments, a peptide comprises about 6 to 12 amino acids in length, e.g., about 9 amino acids in length. In some embodiments, a peptide comprises

about 5 to 10 amino acids in length, e.g., about 7 amino acids in length. In some embodiments, a peptide comprises about 7 to 11 amino acids in length, e.g., about 8 amino acids in length. In some embodiments, a peptide comprises about 4 to 9 amino acids in length, e.g., about 6 amino acids in length.

[0051] In some embodiments a peptide may comprise a sequence as set forth in Table 1 (e.g., comprising the amino acid sequence of any one of SEQ ID NOs: 1100-1658). In some embodiments, the peptide is isolated, e.g., recombinant. In some embodiments a peptide may comprise a sequence as set forth in Tables 37, 38, or 49-52.

Table 1. Exemplary Peptide Sequences

Peptide Sequence	SEQ ID NO:	Peptide Sequence	SEQ ID NO:	Peptide Sequence	SEQ ID NO:	Peptide Sequence	SEQ ID NO:
GGSPHR	1410	RQSPHG	1384	LARSPH	1625	SPHNNR	1175
STSPHR	1440	RLSPHA	1537	YRASPH	1626	SPHPGR	1176
QQSPHR	1441	AKSPHW	1538	RSGSPH	1627	SPHRPV	1177
QSSPHR	1442	QRSPHS	1539	WGTSPH	1628	SPHART	1178
QASPHR	1443	HISPHR	1540	RGSSPH	1629	SPHMRA	1179
MSSPHR	1444	RGSPHS	1541	WHSSPH	1630	SPHRTP	1180
RSSPHP	1445	RNSPHH	1542	SRGSPH	1631	SPHYRT	1181
SQSPHR	1446	ALSPHS	1543	IQGSPH	1632	SPHRLG	1182
DSSPHR	1447	QPSPHR	1544	RPGSPH	1633	SPHVRV	1183
NKSPHK	1448	TLSPHV	1545	APASPH	1634	SPHRLM	1184
NGSPHR	1449	VPSPHR	1546	RQGSPH	1635	SPHRNI	1185
NSSPHR	1450	RASPHN	1547	KTMSPH	1636	SPHMKG	1186
YGSPHR	1451	IESPHR	1548	HWSSPH	1637	SPHRPG	1187
SVSPHR	1391	IRSPHP	1549	RYSSPH	1638	SPHSSK	1188
PGSPHR	1452	LYSPHR	1550	RTGSPH	1639	SPHTAR	1189
RGSPHK	1453	VRSPHY	1551	RNNSPH	1640	SPHSRS	1190
NRSPHK	1454	RMSPHV	1552	APRSPH	1641	SPHVKL	1191
ANSPHR	1396	RVSPHP	1553	LRASPH	1642	SPHMSR	1192
HTSPHR	1455	RLSPHH	1554	PPGSPH	1643	SPHKSH	1193
PTSPHR	1456	SVSPHA	1555	ARGSPH	1644	SPHTMR	1194
TGSPHR	1457	EWSPHR	1556	VGGSPH	1645	SPHRPI	1195
GSSPHR	1458	RISPHL	1557	TGRSPH	1646	SPHVRA	1196
LGSPHR	1459	YRSPHQ	1558	WPNSPH	1647	SPHLNR	1197
KGSPHK	1460	TRSPHG	1559	SWKSPH	1648	SPHKLV	1198
LNSPHK	1461	RYSPHT	1427	RSLSPH	1649	SPHLRP	1199
RSSPHY	1462	RLSPHL	1560	MKHSPH	1650	SPHRVL	1200
LASPHR	1431	GASPHS	1561	RSYSPH	1651	SPHGPK	1201
HLSPHR	1406	LRSPHY	1562	SYGSPH	1652	SPHRVM	1202
RESPHR	1463	ITSPHL	1563	RTFSPH	1653	SPHRTG	1203
HSSPHR	1464	GHSPHG	1564	VTGSPH	1654	SPHRAF	1204
MTSPHR	1465	SKSPHS	1565	RTHSPH	1655	SPHRSM	1205
SWSPHR	1466	KLSPHS	1566	AQRSPH	1656	SPHRSV	1206
QTSPHR	1467	RTSPHT	1390	GGRSPH	1657	SPHQQR	1207
SLSPHR	1387	RTSPHW	1411	LWRSPH	1658	SPHIRV	1208
THSPHR	1468	SPHIPR	1567	SPHRGP	1100	SPHLSR	1209
VASPHR	1469	SPHHGR	1102	SPHRGG	1101	SPHSRF	1210
HGSPHR	1470	SPHSGP	1568	SPHHGR	1102	SPHRSS	1211
RMSPHG	1471	SPHRGS	1138	SPHGRS	1103	SPHAIR	1212
ALSPHR	1472	SPHRMN	1569	SPHAMR	1104	SPHNSR	1213
RMSPHP	1473	SPHLRL	1570	SPHAQR	1105	SPHIRP	1214
VHSPHR	1474	SPHLKG	1571	SPHRAT	1106	SPHSRV	1215

RQSPHK	1475	SPHARL	1140	SPHRGI	1107	SPHRPH	1216
RTSPHP	1476	SPHSRA	1572	SPHARV	1108	SPHRPS	1217
YNSPHR	1477	SPHLSR	1209	SPHQSR	1109	SPHKPW	1218
WGSPHS	1407	GSPHKR	1286	SPHARG	1110	SPHHKL	1219
SASPHR	1379	SSPHRH	1573	SPHRGL	1111	SPHGTR	1220
QVSPHR	1478	SSPHKV	1296	SPHRGM	1112	SPHGHR	1221
YRSPHK	1479	TSPHRQ	1358	SPHQVR	1113	SPHRAS	1222
RASPHP	1480	LSPHKR	1574	SPHRSL	1114	SPHKNL	1223
PYSPHR	1481	TSPHRH	1575	SPHGRN	1115	SPHRVP	1224
LSSPHR	1409	GSPHYK	1576	SPHVRQ	1116	SPHVRL	1225
WLSPHR	1482	GSPHRT	1577	SPHQRS	1117	SPHGAK	1226
LSSPHK	1483	TSPHNR	1347	SPHGFR	1118	SPHRST	1227
VSSPHR	1484	TSPHTK	1578	SPHRTA	1119	SPHSRT	1228
RLSPHY	1485	GSPHSR	1297	SPHGKW	1120	SPHGSW	1229
RMSPHS	1486	SSPHAR	1579	SPHSRL	1121	SPHRLH	1230
RSSPHW	1487	SSPHSR	1291	SPHTRP	1122	SPHKRS	1231
RHSPHK	1488	LSPHRG	1580	SPHGRG	1123	SPHLRI	1232
IHSPHK	1489	GSPHAR	1581	SPHSRM	1124	SPHYKM	1233
NASPHR	1490	SSPHMK	1582	SPHRAA	1125	SPHMRV	1234
DVSPHR	1491	TSPHKP	1583	SPHGGK	1126	SPHHSY	1235
RASPHY	1492	ASPHRI	1584	SPHGRL	1127	SPHKYR	1236
RNSPHG	1493	WSPHVK	1585	SPHSRP	1128	SPHKFK	1237
HVSPHK	1494	LSPHGR	1586	SPHANR	1129	SPHLKT	1238
ARSPHY	1495	MSPHSR	1311	SPHAVR	1130	SPHVKN	1239
RLSPHT	1496	TSPHYR	1587	SPHRYT	1131	SPHLRA	1240
RSSPHA	1423	RSPHSY	1588	SPHRSF	1132	SPHHRA	1241
IQSPHR	1497	HSPHRG	1589	SPHRAG	1133	SPHYGR	1242
IRSPHK	1498	GSPHSK	1590	SPHRAQ	1134	SPHVGR	1243
ERSPHR	1499	RSPHKF	1591	SPHSPR	1135	SPHVRP	1244
RFSPHS	1500	GSPHRL	1305	SPHRAP	1136	SPHVRT	1245
LGSPHS	1501	MSPHHK	1592	SPHPSR	1137	SPHQRM	1246
RLSPHG	1502	TSPHAR	1593	SPHRGS	1138	SPHLGR	1247
RTSPHS	1503	HSPHRV	1365	SPHRMV	1139	SPHKRM	1248
TTSPHS	1504	ASPHSR	1594	SPHARL	1140	SPHMVR	1249
SRSPHN	1505	MSPHMR	1595	SPHRTS	1141	SPHGPF	1250
RGSPHL	1506	SSPHYH	1596	SPHHKP	1142	SPHMRN	1251
WTSPHS	1507	TSPHQR	1597	SPHGKF	1143	SPHVRS	1252
PFSPHG	1508	ASPHGK	1349	SPHARP	1144	SPHKRT	1253
HRSPHP	1509	ISPHAK	1598	SPHARQ	1145	SPHPGK	1254
VYSPHS	1510	ISPHQR	1599	SPHSGR	1146	SPHTGR	1255
RMSPHQ	1511	QSPHVR	1600	SPHTYR	1147	SPHVQR	1256
PRSPHG	1512	LSPHAR	1601	SPHHAR	1148	SPHNAR	1257
LRSPHS	1513	LSPHRS	1602	SPHRVS	1149	SPHWPK	1258
LASPHK	1514	MSPHRH	1603	SPHGNR	1150	SPHIAK	1259
WASPHR	1515	SSPHVR	1338	SPHSRQ	1151	SPHAKF	1260
RGSPHQ	1516	VSPHKQ	1604	SPHGRV	1152	SPHARA	1261
WRSPHG	1517	LSPHTK	1605	SPHSIR	1153	SPHMRF	1262
VRSPHS	1518	VSPHRS	1320	SPHRHT	1154	SPHTRF	1263
RTSPHL	1434	HSPHSR	1606	SPHPRP	1155	SPHSRI	1264
PRSPHM	1519	NSPHGK	1607	SPHRPM	1156	SPHPIR	1265
VLSPHR	1520	TSPHVR	1608	SPHRQP	1157	SPHHMR	1266
MESPHR	1521	GSPHRN	1321	SPHSRH	1158	SPHMQR	1267
RQSPHI	1522	LSPHLG	1609	SPHGPR	1159	SPHSAY	1268
GTSPHA	1523	ESPHRP	1610	SPHRLL	1160	SPHVSK	1269
SVSPHQ	1524	RSPHDR	1611	SPHMHR	1161	SPHMRS	1270
PLSPHA	1525	RSPHLN	1612	SPHKVR	1162	SPHERF	1271
PRSPHA	1526	RPSSPH	1613	SPHRAV	1163	SPHLRG	1272
PRSPHT	1527	LHGSPH	1614	SPHTRT	1164	SPHHYA	1273

STSPHS	1528	RLGSPH	1615	SPHGYR	1165	SPHAGP	1274
EKSPHR	1529	HYSSPH	1616	SPHAKS	1166	SPHPKA	1275
SRSPHA	1530	RFGSPH	1617	SPHQTK	1167	SPHAHL	1276
TLSPHS	1531	RSASPH	1618	SPHATR	1168	SPHEKW	1277
RSSPHS	1532	VRYSPH	1619	SPHRTV	1169	SPHMPR	1278
ARSPHG	1533	KTASPH	1620	SPHVSR	1170	SPHLVR	1279
LGSPHQ	1534	VGSSPH	1621	SPHGLR	1171	SPHPRT	1280
MSSPHA	1535	HRTSPH	1622	SPHALR	1172	SPHGPA	1281
RTSPHV	1536	TPRSPH	1623	SPHRVT	1173	SPHLPR	1282
QRSPHA	1382	LRSSPH	1624	SPHQRN	1174	SPHDRG	1283
SPHRNG	1732	SSPHRS	1733	NSPHKK	1734	KSPHKT	1735

[0052] In some embodiments, the peptide comprises an amino acid sequence comprising at least 4, 5, or 6 consecutive amino acids from any one of the sequences provided in Tables 1 or 49-52. In some embodiments, the peptide comprises an amino acid sequence comprising at least 4, 5, or 6 consecutive amino acids from any one of SEQ ID NOs: 1110-1735. In some embodiments, the peptide comprises an amino acid sequence comprising at least 5 or 6 consecutive amino acids from any one of SEQ ID NOs: 1110-1735.

[0053] In some embodiments, the peptide comprises an amino acid sequence comprising at least 4, 5, 6, or 7 consecutive amino acids from any one of the sequences provided in Table 51. In some embodiments, the peptides comprises an amino acid sequence comprising at least 4, 5, 6, or 7 consecutive amino acids from any one of SEQ ID NOs: 1665-1685. In some embodiments, the peptide comprises the amino acid sequence of any one of SEQ ID NOs: 1665-1685.

[0054] In some embodiments, the peptide comprises an amino acid sequence comprising at least 4, 5, 6, 7, 8, or 9 consecutive amino acids from any one of the sequences provided in Table 52. In some embodiments, the peptides comprises an amino acid sequence comprising at least 4, 5, 6, 7, 8, or 9 consecutive amino acids from any one of SEQ ID NOs: 1686-1731. In some embodiments, the peptide comprises the amino acid sequence of any one of SEQ ID NOs: 1686-1731.

[0055] In some embodiments, the 3 consecutive amino acids comprise SPH. In some embodiments, the 4 consecutive amino acids comprise SPHS (SEQ ID NO: 4700). In some embodiments, the 5 consecutive amino acids comprise SPHSK (SEQ ID NO: 4701). In some embodiments, the 6 consecutive amino acids comprise SPHSKA (SEQ ID NO: 941).

[0056] In some embodiments, 3 consecutive amino acids comprise HDS. In some embodiments, the 4 consecutive amino acids comprise HDSP (SEQ ID NO: 4702). In some embodiments, the 5 consecutive amino acids comprise HDSPH (SEQ ID NO: 4703). In some embodiments, the 6 consecutive amino acids comprise HDSPHK (SEQ ID NO: 2).

[0057] In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the amino acid sequence of any one of the sequences provided in Tables 1, 37, 38 or 49-52. In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, relative to

the amino acid sequence of any one of the sequences provided in Tables 1, 37, 38 or 49-52. In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the amino acid sequence of any one of SEQ ID NOs: 1100-1735. In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of SEQ ID NOs: 1100-1735.

[0058] In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the amino acid sequence of SPHSKA (SEQ ID NO: 941). In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four different amino acids relative to the amino acid sequence of SPHSKA (SEQ ID NO: 941).

[0059] In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the amino acid sequence of HDSPHK (SEQ ID NO: 2). In some embodiments, the peptide comprises an amino acid sequence comprising at least one, two, or three but no more than four different amino acids relative to the amino acid sequence of HDSPHK (SEQ ID NO: 2).

[0060] In some embodiments, the peptide comprises the amino acid sequence of any of the sequences provided in Tables 1, 37, 38 or 49-52. In some embodiments, the peptide comprises the amino acid sequence of any of SEQ ID NOs: 1100-1735.

[0061] In some embodiments, the peptide comprises the amino acid sequence of any of SEQ ID NOs: 1102, 1138, 1140, 1209, 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, 1379, 1382, 1384, 1387, 1390, 1391, 1396, 1406, 1407, 1409, 1410, 1411, 1423, 1427, 1431, 1434, 1440-1571, or 1573-1658. In some embodiments, the peptide comprises the amino acid sequence of any one of SEQ ID NOs: 1379, 1387, 1391, 1396, 1406, 1409, 1410, 1431, 1440-1444, 1446-1461, 1463-1470, 1472, 1474, 1475, 1477-1479, 1481-1484, 1488-1491, 1494, 1497-1499, 1514, 1515, 1520, 1521, 1529, 1540, 1544, 1546, 1548, 1550, or 1556. In some embodiments, the peptide comprises the amino acid sequence of any one of SEQ ID NOs: 1382, 1384, 1390, 1407, 1411, 1423, 1427, 1434, 1445, 1462, 1471, 1473, 1476, 1480, 1485-1487, 1492, 1493, 1495, 1496, 1500-1513, 1516-1519, 1522-1528, 1530-1539, 1541-1543, 1545, 1547, 1549, 1551-1555, or 1557-1566. In some embodiments, the peptide comprises the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1567, 1568, 1569, 1570, or 1571. In some embodiments, the peptide comprises the amino acid sequence of any one of SEQ ID NOs: 1286, 1291, 1296, 1297, 1305, 1311,

1320, 1321, 1338, 1347, 1349, 1358, 1365, or 1573-1612. In some embodiments, the peptide comprises the amino acid sequence of any one of SEQ ID NOs: 1613-1658.

[0062] In some embodiments, the peptide comprises the amino acid sequence of any one of SEQ ID NOs: 1100-1439. In some embodiments, the peptide comprises the amino acid sequence of any one of SEQ ID NOs: 1100-1283. In some embodiments, the peptide comprises the amino acid sequence of any one of SEQ ID NOs: 1284-1376. In some embodiments, the peptide comprises the amino acid sequence of any one of SEQ ID NOs: 1377-1437. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 1438 or 1439.

[0063] In some embodiments, a peptide described herein is fused or coupled, e.g., conjugated, to an active agent. In some embodiments, the active agent is a therapeutic agent. In some embodiments, the agent is a therapeutic agent. In some embodiments, the active agent comprises a therapeutic protein, an antibody molecule, an enzyme, one or more components of a genome editing system, an Fc polypeptide fused or coupled (e.g., covalently or non covalently) to a therapeutic agent, and/or an RNAi agent (e.g., a dsRNA, antisense oligonucleotide (ASO), siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA). In some embodiments, the therapeutic agent is an antibody. In some embodiments, the peptide is fused or coupled, e.g., conjugated (e.g., directly or indirectly) to the Fc region of the antibody, e.g., at the C-terminus of the Fc region or the N-terminus of the Fc region. In some embodiments, the therapeutic agent is an RNAi agent. In some embodiments, the RNAi agent is a siRNA or an ASO. In some embodiments, the ASO or siRNA comprises at least one (e.g., one or more or all) modified nucleotides. In some embodiments, the peptide is fused or coupled, e.g., conjugated (e.g., directly or indirectly via a linker), to at least one strand of the RNAi agent. In some embodiments, the peptide is conjugated, e.g., directly or indirectly via a linker, to the C-terminus of at least one strand of the RNAi agent. In some embodiments, the peptide is conjugated, e.g., directly or indirectly via a linker, to an internal nucleotide of at least one strand of the RNAi agent. In some embodiments, the at least one strand is the sense strand. In some embodiments, the therapeutic agent modulates, e.g., inhibits, decreases, or increases, expression of, a CNS related gene, mRNA, and/or protein.

[0064] In some embodiments, the active agent is a diagnostic agent. In some embodiments, the diagnostic agent is or comprises an imaging agent (e.g., a protein or small molecule compound coupled to a detectable moiety). In some embodiments, the imaging agent comprises a PET or MRI ligand, or an antibody molecule coupled to a detectable moiety. In some embodiments, the detectable moiety is or comprises a radiolabel, a fluorophore, a chromophore, or an affinity tag. In some embodiments, the radiolabel is or comprises tc99m, iodine-123, a spin label, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen- 15, oxygen-17, gadolinium, manganese, or iron. In some embodiments, the active agent is a small molecule. In some embodiments, the active agent is a

ribonucleic acid complex (e.g., a Cas9/gRNA complex), a plasmid, a closed-end DNA, a circ-RNA, or an mRNA.

[0065] In some embodiments, at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides are fused or coupled, e.g., conjugated, to an active agent, e.g., a therapeutic agent or a diagnostic agent. In some embodiments, the at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides comprise the same amino acid sequence. In some embodiments, the at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides comprise different amino acid sequences. In some embodiments, the at least 1-5, e.g., at least 1, 2, 3, 4, or 5, peptides are present in tandem (e.g., connected directly or indirectly via a linker) or in a multimeric configuration. In some embodiments, the peptide comprises an amino acid sequence of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 20, 25, 30, or 35 amino acids in length.

[0066] In some embodiments, the peptide covalently linked, e.g., directly or indirectly via a linker, to the active agent. In some embodiments, the peptide is conjugated to the active agent via a linker. In some embodiments, the linker is a cleavable linker or a non-cleavable linker. In some embodiments, the cleavable linker is a pH sensitive linker or an enzyme sensitive linker. In some embodiments, the pH sensitive linker comprises a hydrazine/hydrazone linker or a disulfide linker. In some embodiments, the enzyme sensitive linker comprises a peptide based linker, e.g., a peptide linker sensitive to a protease (e.g., a lysosomal protease); or a beta-glucuronide linker. In some embodiments, the non-cleavable linker is a linker comprising a thioether group or a maleimidocaproyl group. In some embodiments, the peptide and the active agent are fused or coupled post-translationally, e.g., using click chemistry. In some embodiments, the peptide and the active agent are fused or couple via chemically induced dimerization. In some embodiments, the peptide is present N-terminal relative to the active agent. In some embodiments, the peptide is present C-terminal relative to the active agent.

[0067] In some embodiments, the peptide is present or coupled to a carrier. In some embodiments, the carrier comprises an exosome, a microvesicle, or a lipid nanoparticle (LNP). In some embodiments, the carrier comprises a therapeutic agent (e.g., an RNAi agent (e.g., an dsRNA, a siRNA, a shRNA, a pre-miRNA, a pri-miRNA, a miRNA, a stRNA, a lncRNA, a piRNA, an antisense oligonucleotide agent (ASO), or a snoRNA), an mRNA, a ribonucleoprotein complex (e.g., a Cas9/gRNA complex), or a circRNA). In some embodiments, the peptide is present on the surface of the carrier. In some embodiments, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the surface of the carrier comprises at least 1-5, e.g., at least 1, 2, 3, 4, or 5 peptides described herein.

[0068] The present disclosure also provides a nucleic acid or polynucleotide encoding any of the peptides described herein and AAV capsid variants, AAV particles, vectors, and cells comprising the same.

AAV Capsid Variant

[0069] In some embodiments, an AAV particle described herein comprises an AAV capsid variant, e.g., an AAV capsid variant described herein (e.g., an AAV capsid variant comprising a peptide described herein). In some embodiments, an AAV capsid variant comprises a peptide as set forth in any of Tables 1, 37, 38 or 49-52.

[0070] In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence comprising at least 4, 5, or 6 consecutive amino acids from any one of the sequences provided in Tables 1, 37, 38 or 49-52. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least 4, 5, or 6 consecutive amino acids from any one of SEQ ID NOs: 1110-1735. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least 5 or 6 consecutive amino acids from any one of SEQ ID NOs: 1110-1735. In some embodiments, the amino acid sequence is present in loop IV. In some embodiments, the amino acid sequence is present immediately subsequent to position 453 or 455, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138, 981, or 982. In some embodiments, the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 981. In some embodiments, the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 981. In some embodiments, the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 982. In some embodiments, the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 982. In some embodiments, the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 138.

[0071] In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least 4, 5, or 6 consecutive amino acids from any one of the sequences provided in Tables 1 or 49-52. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least 4, 5, or 6 consecutive amino acids from any one of SEQ ID NOs: 1110-1735. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least 5 or 6 consecutive amino acids from any one of SEQ ID NOs: 1110-1735.

[0072] In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least 4, 5, 6, or 7 consecutive amino acids from any one of the sequences provided in Table 51. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least 4, 5, 6, or 7 consecutive amino acids from any one of SEQ ID NOs: 1665-1685. In

some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 1665-1685.

[0073] In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least 4, 5, 6, 7, 8, or 9 consecutive amino acids from any one of the sequences provided in Table 52. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least 4, 5, 6, 7, 8, or 9 consecutive amino acids from any one of SEQ ID NOs: 1686-1731. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 1686-1731.

[0074] In some embodiments, the AAV capsid variant comprises the amino acid sequence of any of SEQ ID NOs: 1102, 1138, 1140, 1209, 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, 1379, 1382, 1384, 1387, 1390, 1391, 1396, 1406, 1407, 1409, 1410, 1411, 1423, 1427, 1431, 1434, 1440-1571, or 1573-1658. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 1379, 1387, 1391, 1396, 1406, 1409, 1410, 1431, 1440-1444, 1446-1461, 1463-1470, 1472, 1474, 1475, 1477-1479, 1481-1484, 1488-1491, 1494, 1497-1499, 1514, 1515, 1520, 1521, 1529, 1540, 1544, 1546, 1548, 1550, or 1556. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 1382, 1384, 1390, 1407, 1411, 1423, 1427, 1434, 1445, 1462, 1471, 1473, 1476, 1480, 1485-1487, 1492, 1493, 1495, 1496, 1500-1513, 1516-1519, 1522-1528, 1530-1539, 1541-1543, 1545, 1547, 1549, 1551-1555, or 1557-1566. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1567, 1568, 1569, 1570, or 1571. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, or 1573-1612. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 1613-1658. In some embodiments, the amino acid sequence is present in loop IV. In some embodiments, the amino acid sequence is present immediately subsequent to position 453 or 455, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138, 981, or 982. In some embodiments, the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 982. In some embodiments, the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 982. In some embodiments, the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 138.

[0075] In some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 1100-1439. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 1100-1283. In some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 1284-1376. In some embodiments,

the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 1377-1437. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 1438 or 1439. In some embodiments, the amino acid sequence is present in loop IV. In some embodiments, the amino acid sequence is present immediately subsequent to position 453 or 455, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138, 981, or 982. In some embodiments, the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 981. In some embodiments, the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 981. In some embodiments, the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 138.

[0076] In some embodiments, the 3 consecutive amino acids comprise SPH. In some embodiments, the 4 consecutive amino acids comprise SPHS (SEQ ID NO: 4700). In some embodiments, the 5 consecutive amino acids comprise SPHSK (SEQ ID NO: 4701). In some embodiments, the 6 consecutive amino acids comprise SPHSKA (SEQ ID NO: 941).

[0077] In some embodiments, 3 consecutive amino acids comprise HDS. In some embodiments, the 4 consecutive amino acids comprise HDSP (SEQ ID NO: 4702). In some embodiments, the 5 consecutive amino acids comprise HDSPH (SEQ ID NO: 4703). In some embodiments, the 6 consecutive amino acids comprise HDSPHK (SEQ ID NO: 2).

[0078] In some embodiments, an AAV capsid variant described herein comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the amino acid sequence of any one of the sequences provided in Tables 1, 37, 38 or 49-52 In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of the sequences provided in Tables 1, 37, 38 or 49-52. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the amino acid sequence of any one of SEQ ID NOs: 1100-1735. In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, relative to the amino acid sequence of any one of SEQ ID NOs: 1100-1735. In some embodiments, the amino acid sequence is present in loop IV. In some embodiments, the amino acid sequence is present immediately subsequent to position 453 or 455, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138, 981, or 982. In some embodiments, the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 981. In some embodiments, the amino acid sequence is present immediately subsequent to

position 455, numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 981. In some embodiments, the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 982. In some embodiments, the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 982. In some embodiments, the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 138.

[0079] In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the amino acid sequence of SPHSKA (SEQ ID NO: 941). In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least one, two, or three, but no more than four different amino acids from the amino acid sequence of SPHSKA (SEQ ID NO: 941).

[0080] In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the amino acid sequence of HDSPHK (SEQ ID NO: 2). In some embodiments, the AAV capsid variant comprises an amino acid sequence comprising at least one, two, or three, but no more than four different amino acids that relative to the amino acid sequence of HDSPHK (SEQ ID NO: 2).

[0081] In some embodiments, the AAV capsid variant, comprises the amino acid sequence of any of the sequences provided in Tables 1, 37, 38 or 49-52. In some embodiments, the peptide comprises the amino acid sequence of any of SEQ ID NOs: 1100-1735. In some embodiments, the amino acid sequence is present in loop IV. In some embodiments, the amino acid sequence is present immediately subsequent to position 453 or 455, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138, 981, or 982. In some embodiments, the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 981. In some embodiments, the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 981. In some embodiments, the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 982. In some embodiments, the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138. In some embodiments, the amino acid sequence is present at positions 454-459, numbered according to SEQ

ID NO: 982. In some embodiments, the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 138.

[0082] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SPHSKA (SEQ ID NO: 941), wherein the amino acid sequence is present immediately subsequent to position 455, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SPHSKA (SEQ ID NO: 941), wherein the amino acid sequence is present immediately subsequent to position 455, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 981.

[0083] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of HDSPHK (SEQ ID NO: 2), wherein the amino acid sequence is present immediately subsequent to position 453, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of HDSPHK (SEQ ID NO: 2), wherein the amino acid sequence is present immediately subsequent to position 453, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 982.

[0084] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 1659. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 1659, wherein the amino acid sequence is present immediately subsequent to position 453 (e.g., at positions 454-459), numbered according to the amino acid sequence of SEQ ID NO: 982.

[0085] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 1660. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 1660, wherein the amino acid sequence is present immediately subsequent to position 453 (e.g., at positions 454-459), numbered according to the amino acid sequence of SEQ ID NO: 982.

[0086] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 1661. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 1661, wherein the amino acid sequence is present immediately subsequent to position 455 (e.g., at positions 456-461), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 981.

[0087] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 1662. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 1662, wherein the amino acid sequence is present immediately subsequent to position 455 (e.g., at positions 456-461), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 981.

[0088] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 1663. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 1663, wherein the amino acid sequence is present immediately subsequent to position 455 (e.g., at positions 456-461), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 981.

[0089] In some embodiments, an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 1664. In some embodiments, the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 1664, wherein the amino acid sequence is present immediately subsequent to position 455 (e.g., at positions 456-461), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 981.

[0090] In some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 1665-1685, wherein the amino acid sequence is present immediately subsequent to position 453 (e.g., at positions 454-460), numbered according to the amino acid sequence of SEQ ID NO: 982 or 138.

[0091] In some embodiments, the AAV capsid variant comprises the amino acid sequence of any one of SEQ ID NOs: 1686-1731, wherein the amino acid sequence is present immediately subsequent to position 453 (e.g., at positions 454-462), numbered according to the amino acid sequence of SEQ ID NO: 982 or 138.

[0092] In some embodiments, the AAV capsid variant, further comprises a substitution at position K449, e.g., a K449R substitution, numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant, further comprises an amino acid other than K at position 449 (e.g., R), relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises an R at position 449, relative to a reference sequence numbered according to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises a modification, e.g., an insertion, substitution, and/or deletion in loop I, II, VI, and/or VIII.

[0093] In some embodiments, the AAV capsid variant, further comprises an amino acid sequence comprising at least one, two or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but not more than 30, 20 or 10 modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, of the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant, further comprises an amino acid sequence comprising at least one, two or three, but not more than 30, 20 or 10 amino acids that differ from the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid variant further comprises the amino acid sequence of SEQ ID NO: 138, or an amino acid sequence with at least 70% (e.g., at least about 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto.

[0094] In some embodiments, the AAV capsid variant further comprises (a) a VP1 protein comprising the amino acid sequence of SEQ ID NO: 138; (b) a VP2 protein comprising the amino acid sequence of positions 138-736 of SEQ ID NO: 138; (c) a VP3 protein comprising the amino acid sequence of positions 203-736 of SEQ ID NO: 138; or (d) an amino acid sequence with at least 70% (e.g., at least about 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity to any of the amino acid sequences in (a)-(c), an amino acid sequence comprising at least one, two or three, but not more than 30, 20 or 10 different amino acids relative to any of the amino acid sequences in (a)-(c), or an amino acid sequence comprising at least one, two or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but not more than 30, 20 or 10 modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to any of the amino acid sequences in (a)-(c).

[0095] In some embodiments, the AAV capsid variant further comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 137, or a sequence with at least 70% (e.g., at least about 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto. In some embodiments, the AAV capsid variant further comprises an amino acid sequence encoded by a nucleotide sequence comprising at least one, two or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but not more than 30, 20 or 10 modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 137. In some embodiments, the AAV capsid variant further comprises an amino acid sequence encoded by a nucleotide sequence comprising at least one, two or three, but not more than 30, 20 or 10 different nucleotides, relative to the amino acid sequence of SEQ ID NO: 137.

[0096] In some embodiments, the nucleotide sequence encoding the AAV capsid variant further comprises the nucleotide sequence of SEQ ID NO: 137, or a sequence with at least 70% (e.g., at least about 80, 85, 90, 95, 96, 97, 98, or 99%) sequence identity thereto. In some embodiments, the nucleotide sequence encoding the AAV capsid variant further comprises a nucleotide sequence comprising at least one, two or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but not more than 30, 20 or 10 modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 137. In some embodiments, the nucleotide sequence encoding the AAV capsid variant further comprises a nucleotide sequence comprising at least one, two or three, but not more than 30, 20 or 10 different nucleotides, relative to the amino acid sequence of SEQ ID NO: 137.

[0097] In some embodiments, an AAV capsid variant of the present disclosure comprises an amino acid sequence as described herein, e.g., an amino acid sequence of an AAV capsid variant of TTM-001 or TTM-002, e.g., as described in Table 4.

[0098] In some embodiments, an AAV capsid variant described herein comprises a VP1, VP2, and/or VP3 protein comprising an amino acid sequence described herein, e.g., an amino acid sequence of an AAV capsid variant of TTM-001 or TTM-002, e.g., as described in Table 4.

Table 4. Exemplary full length capsid amino acid sequences

Name and Annotation	SEQ ID NO:	Amino Acid Sequence
TTM-001 6mer peptide underlined, starts at position 456 (immediately subsequent to position 455); 742 aa	981	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPG NGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGG NLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAK KRLNFGQTGDTESVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSS SGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTP WGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIAN NLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRS SFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLS KTINGSG**SPHSKA**QNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNN SEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVD ADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQGILPGMVWQDR DVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDK LNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGVYS EPRPIGTRYLTRNL
TTM-002 6mer peptide underlined, starts at position 454 (immediately subsequent to position 453); 742 aa	982	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPG NGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGG NLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAK KRLNFGQTGDTESVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSS SGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTP WGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIAN NLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRS SFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLS KTING**HDSPHK**SGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNN SEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVD ADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQGILPGMVWQDR DVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDK LNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGVYS EPRPIGTRYLTRNL
TTM-003 6mer peptide underlined, starts at position 456 (immediately subsequent to position 455); modifications at positions 451, 452, and 453 underlined; 742 aa	36	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPG NGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGG NLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAK KRLNFGQTGDTESVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSS SGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTP WGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIAN NLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRS SFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLS KT**ERV**SG**SPHSKA**QNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNN SEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVD ADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQGILPGMVWQDR DVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDK LNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGVYS EPRPIGTRYLTRNL
TTM-006 6mer peptide underlined, starts at position 456 (immediately subsequent to position 455); modifications at	39	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPG NGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGG NLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAK KRLNFGQTGDTESVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSS SGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTP WGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIAN NLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRS SFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLS KT**EKM**SG**SPHSKA**QNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNN

positions 451, 452, and 453 underlined; 742 aa		SEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVD ADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQGILPGMVWQDR DVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDK LNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGVYS EPRPIGTRYLTRNL
TTM-018 6mer peptide underlined, starts at position 456 (immediately subsequent to position 455); modifications at positions 454, 455, and 464 underlined; 742 aa	51	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPG NGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGG NLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAK KRLNFGQTGDTESVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSS SGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTP WGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIAN NLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRS SFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLS KTING**HDSPHSKA**QN**L**QTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNN SEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVD ADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQGILPGMVWQDR DVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDK LNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGVYS EPRPIGTRYLTRNL
TTM-019 6mer peptide underlined, starts at position 456 (immediately subsequent to position 455); modifications at positions 451, 454, and 455 underlined; 742 aa	52	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPG NGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGG NLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAK KRLNFGQTGDTESVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSS SGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTP WGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIAN NLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRS SFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLS KT**V**NG**HDSPHSKA**QNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNN SEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVD ADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQGILPGMVWQDR DVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDK LNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGVYS EPRPIGTRYLTRNL

[0099] In some embodiments, an AAV capsid variant, described herein has an increased tropism for a CNS cell or tissue, e.g., a brain cell, brain tissue, spinal cord cell, or spinal cord tissue, relative to the tropism of a reference sequence comprising the amino acid sequence of SEQ ID NO: 138. In some embodiments, an AAV capsid variant described herein transduces a brain region, e.g., a midbrain region (e.g., the hippocampus, or thalamus) or the brain stem.

[0100] In some embodiments, an AAV capsid variant described herein shows preferential transduction in a brain region relative to the transduction in the dorsal root ganglia (DRG). In some embodiments, the AAV capsid variant shows preferential transduction in a brain region relative to the transduction in the liver. In some embodiments, the AAV capsid variant shows preferential transduction in a brain region relative to the transduction in the liver and the DRG. In some embodiments, the AAV capsid variant shows preferential transduction in a brain region relative to the transduction in the heart. In some embodiments, the AAV capsid variant shows preferential transduction in a brain region relative to the transduction in the heart and DRG. In some embodiments, the AAV capsid variant shows preferential transduction in a brain region relative to the transduction in the heart, DRG, and liver. In some embodiments, the AAV capsid variant shows

preferential transduction in a brain region and/or a heart region relative to the transduction in the liver and DRG.

[0101] In some embodiments, an AAV capsid variant described herein is capable of transducing non-neuronal cells, e.g., glial cells (e.g., oligodendrocytes or astrocytes). In some embodiments, the AAV capsid variant described herein is capable of transducing neuronal cells and non-neuronal cells, e.g., glial cells (e.g., oligodendrocytes or astrocytes). In some embodiments, the non-neuronal cells are glial cells, oligodendrocytes (e.g., Olig2 positive oligodendrocytes), or astrocytes (e.g., Olig2 positive astrocytes). In some embodiments, the AAV capsid variant is capable of transducing Olig2 positive cells, e.g., Olig2 positive astrocytes or Olig2 positive oligodendrocytes.

[0102] In some embodiments, an AAV capsid variant described herein is capable of binding to a glycosylphosphatidylinositol (GPI) anchored protein, e.g., alkaline phosphatase (ALPL). In some embodiments, the GPI anchored protein is conserved in at least two to three species, e.g., at least three species (e.g., mice, NHPs (e.g., *Macaca fascicularis*), and/or humans). In some embodiments, the GPI anchored protein is present on the surface of a cell in the blood brain barrier. In some embodiments, the GPI anchored protein is ALPL. In some embodiments, the AAV capsid variant is capable of binding N-linked galactose. In some embodiments, binding to ALPL results in increased cellular transduction, e.g., as compared to a reference sequence of SEQ ID NO: 138. In some embodiments, binding to ALPL results in increased crossing of the blood brain barrier, e.g., as compared to a reference sequence of SEQ ID NO: 138. Without wishing to be bound by theory, it is believed in some embodiments, that the binding of the AAV capsid variants described herein to ALPL is part of the mechanism leading to increased crossing of the blood brain barrier relative to the AAV9 control.

[0103] ALPL is part of a family of membrane-bound glycoproteins that hydrolyze monophosphate esters at a high pH (see, e.g., Weiss et al., Isolation and characterization of a cDNA encoding a human liver/bone/kidney-type alkaline phosphatase. Proc. Nat. Acad. Sci., 83: 7182-7186 (1986), the contents of which are hereby incorporated by reference in their entirety). ALPL is highly conserved across humans, mice, and cynomolgus macaques (*Macaca fascicularis*) when compared by sequence alignment (e.g., as shown in **Table 26**). Additionally, in humans ALPL is expressed on endothelial cells and neurons, and at a low level on astrocytes. The highest level of ALPL expression in human is on endothelial cells. In mice, ALPL is more highly expressed on astrocytes, oligodendrocyte progenitor cells (OPCs), and to a lesser extent on endothelial cells. Without wishing to be bound by theory, it is believed in some embodiments that highly conserved nature of the ALPL receptor protein across species is predictive of cross-species compatibility of the AAV capsid variants described herein. Without wishing to be bound by theory, it is believed in some embodiments, that ALPL is upregulated in aging brain (e.g., as described in Yang et al. "Physiological blood–brain

transport is impaired with age by a shift in transcytosis," *Nature*. 2020 583:425-430, the contents of which are hereby incorporated by reference in its entirety).

[0104] In some embodiments, an AAV capsid variant of the present disclosure has decreased tropism for the liver. In some embodiments, an AAV capsid variant comprises a modification, e.g., substitution (e.g., conservative substitution), insertion, or deletion, that results in reduced tropism (e.g., de-targeting) and/or activity in the liver. In some embodiments, the reduced tropism in the liver is compared to an otherwise similar capsid that does not comprise the modification, e.g., a wild-type capsid. In some embodiments, an AAV capsid variant described herein comprises a modification, e.g., substitution (e.g., conservative substitution), insertion, or deletion that results in one or more of the following properties: (1) reduced tropism in the liver; (2) reduced, e.g., de-targeted, expression in the liver; (3) reduced activity in the liver; and/or (4) reduced binding to galactose. In some embodiments, the reduction in any one, or all of properties (1)-(3) is compared to an otherwise similar AAV capsid variant that does not comprise the modification. Exemplary modifications are provided in WO 2018/119330; Pulicherla et al. (2011) *Mol. Ther.* 19(6): 1070-1078; Adachi et al. (2014) *Nature Communications* 5(3075), DOI: 10.1038/ncomms4075; and Bell et al. (2012) *J. Virol.* 86(13): 7326-33; the contents of which are hereby incorporated by reference in their entirety. In some embodiments, the AAV capsid variant comprises a modification e.g., substitution (e.g., conservative substitution), insertion, or deletion, at position N470 (e.g., N470A), D271 (e.g., D271A), N272 (e.g., N272A), Y446 (e.g., Y446A), N498 (e.g., N498Y or N498I), W503 (e.g., W530R or W530A), L620 (e.g., L620F), or a combination thereof, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises one, two, three, four, five or all of an amino acid other than N at position 470 (e.g., A), an amino acid other than D at position 271 (e.g., A), an amino acid other than N at position 272 (e.g., A), an amino acid other than Y at position 446 (e.g., A), and amino acid other than N at position 498 (e.g., Y or I), and amino acid other than W at position 503 (e.g., R or A), and amino acid other than L at position 620 (e.g., F), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises a modification e.g., substitution (e.g., conservative substitution), insertion, or deletion, at position N470 (e.g., N470A), D271 (e.g., D271A), N272 (e.g., N272A), Y446 (e.g., Y446A), and W503 (e.g., W503R or W503A), relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, the AAV capsid variant comprises a modification e.g., substitution (e.g., conservative substitution), insertion, or deletion, at N498 (e.g., N498Y) and L620 (e.g., L620F).

[0105] In some embodiments, an AAV capsid variant comprised herein comprises a modification as described in Adachi et al. (2014) *Nature Communications* 5(3075), DOI: 10.1038/ncomms4075, the contents of which are hereby incorporated by reference in its entirety. Exemplary modifications that alter or do not alter tissue transduction in at least the brain, liver, heart, lung, and/or kidney can be found in Supplementary Data 2 showing the AAV Barcode-Seq data obtained with AAV9-AA-

VBCLib of Adachi et al. (*supra*), the contents of which are hereby incorporated by reference in its entirety.

[0106] In some embodiments, an AAV capsid variant of the present disclosure is isolated, e.g., recombinant. In some embodiments, a polynucleotide encoding an AAV capsid polypeptide, e.g., an AAV capsid variant, of the present disclosure is isolated, e.g., recombinant.

[0107] Also provided herein are polynucleotide sequences encoding any of the AAV capsid variants described above and AAV particles, vectors, and cells comprising the same.

AAV serotypes and capsids

[0108] In some embodiments, an AAV particle of the present disclosure may comprise a capsid protein or variant thereof any natural or recombinant AAV serotype. AAV serotypes may differ in characteristics such as, but not limited to, packaging, tropism, transduction and immunogenic profiles. While not wishing to be bound by theory, it is believed in some embodiments, that the AAV capsid protein, e.g., an AAV capsid variant, can modulate, e.g., direct, AAV particle tropism to a particular tissue.

[0109] In some embodiments, an AAV capsid variant described herein allows for blood brain barrier penetration following intravenous administration. In some embodiments, the AAV capsid variant allows for blood brain barrier penetration following intravenous administration, focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration. In some embodiments the AAV capsid variant allows for increased distribution to a brain region. In some embodiments, the brain region comprises a frontal cortex, sensory cortex, motor cortex, caudate, dentate nucleus, cerebellar cortex, cerebral cortex, brain stem, hippocampus, thalamus, putamen, or a combination thereof. In some embodiments, the AAV capsid variant allows for preferential transduction in a brain region relative to the transduction in the dorsal root ganglia (DRG). In some embodiments, the AAV capsid variant allows for transduction in a non-neuronal cell, e.g., a glial cell (e.g., an astrocyte, an oligodendrocyte, or a combination thereof).

[0110] In some embodiments, the initiation codon for translation of the AAV VP1 capsid protein, e.g., a capsid variant, described herein may be CTG, TTG, or GTG as described in US Patent No. US8163543, the contents of which are herein incorporated by reference in its entirety.

[0111] The present disclosure refers to structural capsid proteins (including VP1, VP2 and VP3) which are encoded by capsid (Cap) genes. These capsid proteins form an outer protein structural shell (e.g., capsid) of a viral vector such as AAV. VP capsid proteins synthesized from Cap polynucleotides generally include a methionine as the first amino acid in the peptide sequence (Met1), which is associated with the start codon (AUG or ATG) in the corresponding Cap nucleotide sequence. However, it is common for a first-methionine (Met1) residue or generally any first amino acid (AA1) to be cleaved off after or during polypeptide synthesis by protein processing enzymes such as Met-

aminopeptidases. This "Met/AA-clipping" process often correlates with a corresponding acetylation of the second amino acid in the polypeptide sequence (e.g., alanine, valine, serine, threonine, etc.). Met-clipping commonly occurs with VP1 and VP3 capsid proteins but can also occur with VP2 capsid proteins.

[0112] Where the Met/AA-clipping is incomplete, a mixture of one or more (one, two or three) VP capsid proteins comprising the viral capsid may be produced, some of which may include a Met1/AA1 amino acid (Met+/AA+) and some of which may lack a Met1/AA1 amino acid as a result of Met/AA-clipping (Met-/AA-). For further discussion regarding Met/AA-clipping in capsid proteins, see Jin, et al. Direct Liquid Chromatography/Mass Spectrometry Analysis for Complete Characterization of Recombinant Adeno-Associated Virus Capsid Proteins. *Hum Gene Ther Methods.* 2017 Oct. 28(5):255-267; Hwang, et al. N-Terminal Acetylation of Cellular Proteins Creates Specific Degradation Signals. *Science.* 2010 February 19. 327(5968): 973–977; the contents of which are each incorporated herein by reference in its entirety.

[0113] According to the present disclosure, references to capsid proteins, e.g., AAV capsid variants, is not limited to either clipped (Met-/AA-) or unclipped (Met+/AA+) and may, in context, refer to independent capsid proteins, viral capsids comprised of a mixture of capsid proteins, and/or polynucleotide sequences (or fragments thereof) which encode, describe, produce or result in capsid proteins of the present disclosure. A direct reference to a capsid protein or capsid polypeptide (such as VP1, VP2 or VP2) may also comprise VP capsid proteins which include a Met1/AA1 amino acid (Met+/AA+) as well as corresponding VP capsid proteins which lack the Met1/AA1 amino acid as a result of Met/AA-clipping (Met-/AA-).

[0114] Further according to the present disclosure, a reference to a specific SEQ ID NO: (whether a protein or nucleic acid) which comprises or encodes, respectively, one or more capsid proteins which include a Met1/AA1 amino acid (Met+/AA+) should be understood to teach the VP capsid proteins which lack the Met1/AA1 amino acid as upon review of the sequence, it is readily apparent any sequence which merely lacks the first listed amino acid (whether or not Met1/AA1).

[0115] As a non-limiting example, reference to a VP1 polypeptide sequence which is 736 amino acids in length, and which includes a "Met1" amino acid (Met+) encoded by the AUG/ATG start codon may also be understood to teach a VP1 polypeptide sequence which is 735 amino acids in length, and which does not include the "Met1" amino acid (Met-) of the 736 amino acid Met+ sequence. As a second non-limiting example, reference to a VP1 polypeptide sequence which is 736 amino acids in length, and which includes an "AA1" amino acid (AA1+) encoded by any NNN initiator codon may also be understood to teach a VP1 polypeptide sequence which is 735 amino acids in length, and which does not include the "AA1" amino acid (AA1-) of the 736 amino acid AA1+ sequence.

[0116] References to viral capsids formed from VP capsid proteins (such as reference to specific AAV capsid serotypes), can incorporate VP capsid proteins which include a Met1/AA1 amino acid (Met+/AA1+), corresponding VP capsid proteins which lack the Met1/AA1 amino acid as a result of Met/AA1-clipping (Met-/AA1-), and combinations thereof (Met+/AA1+ and Met-/AA1-).

[0117] As a non-limiting example, an AAV capsid serotype can include VP1 (Met+/AA1+), VP1 (Met-/AA1-), or a combination of VP1 (Met+/AA1+) and VP1 (Met-/AA1-). An AAV capsid serotype can also include VP3 (Met+/AA1+), VP3 (Met-/AA1-), or a combination of VP3 (Met+/AA1+) and VP3 (Met-/AA1-); and can also include similar optional combinations of VP2 (Met+/AA1) and VP2 (Met-/AA1-).

Additional AAV Sequences

[0118] In some embodiments, the AAV capsid variant, comprises immediately subsequent to position 448, 449, 452, 453, 455, numbered relative to SEQ ID NO: 138 or corresponding to equivalent positions in any other AAV serotype (e.g., AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, SEQ ID NO: 1, SEQ ID NO: 11, PHP.N, PHP.B, or an AAV serotype as provided in Table 6 of WO 2021/230987 (the contents of which are hereby incorporated by reference in their entirety)), at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 consecutive amino acids of any of amino acid sequence provided in Tables 1, 37, 38, or 49-52. In some embodiments, the at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 consecutive amino acids of any of amino acid sequence provided in Tables 1, 37, 38, or 49-52 replaces at least one, two, three, four, five, six, seven, eight, nine, ten, or all of positions K449, T450, I451, N452, G453, S454, G455, Q456, N457, Q458, and/or Q459, numbered according to SEQ ID NO: 138 or corresponding to equivalent positions in any other AAV serotype (e.g., AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, SEQ ID NO: 1, SEQ ID NO: 11, PHP.N, PHP.B, or an AAV serotype as provided in Table 6 of WO 2021/230987 (the contents of which are hereby incorporated by reference in their entirety). In some embodiments, the at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 consecutive amino acids of any of amino acid sequence provided in Tables 1, 37, 38, or 49-52 replaces positions S454, G455, or both positions S454 and G455, numbered according to SEQ ID NO: 138 or corresponding to equivalent positions in any other AAV serotype (e.g., AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, SEQ ID NO: 1, SEQ ID NO: 11, PHP.N, PHP.B, or an AAV serotype as provided in Table 6 of WO 2021/230987 (the contents of which are hereby incorporated by reference in their entirety). In some embodiments, the AAV capsid variant comprises an amino acid other than the wild-type, e.g., native, amino acid, at one, two, three, four, five, six, seven, eight, nine or all of positions T450, I451, N452, G453, S454, G455, Q456, N457, Q458, and/or Q459, numbered according to SEQ ID NO: 138 or corresponding to equivalent positions in any other AAV serotype (e.g., AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh10,

AAVrh32.33, AAVrh74, SEQ ID NO: 1, SEQ ID NO: 11, PHP.N, PHP.B, or an AAV serotype as provided in Table 6 of WO 2021/230987 (the contents of which are hereby incorporated by reference in their entirety). In some embodiments, the AAV capsid variant comprises an amino acid other than the wild-type, e.g., native, amino acid, at position S454, G455, or both positions S454 and G455, numbered according to SEQ ID NO: 138 or corresponding to equivalent positions in any other AAV serotype (e.g., AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, SEQ ID NO: 1, SEQ ID NO: 11, PHP.N, PHP.B, or an AAV serotype as provided in Table 6 of WO 2021/230987 (the contents of which are hereby incorporated by reference in their entirety)). In some embodiments, the AAV capsid variant comprises a modification, e.g., substitution, at one, two, three, four, five, six, seven, eight, nine, ten or all of positions K449, T450, I451, N452, G453, S454, G455, Q456, N457, Q458, and/or Q459, numbered according to SEQ ID NO: 138 or corresponding to equivalent positions in any other AAV serotype (e.g., AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, SEQ ID NO: 1, SEQ ID NO: 11, PHP.N, PHP.B, or an AAV serotype as provided in Table 6 of WO 2021/230987 (the contents of which are hereby incorporated by reference in their entirety). In some embodiments, the AAV capsid variant comprises a modification, e.g., substitution, at position S454, G455, or both positions S454 and G455, numbered according to SEQ ID NO: 138 or corresponding to equivalent positions in any other AAV serotype (e.g., AAV1, AAV2, AAV3, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, SEQ ID NO: 1, SEQ ID NO: 11, PHP.N, PHP.B, or an AAV serotype as provided in Table 6 of WO 2021/230987 (the contents of which are hereby incorporated by reference in their entirety).

[0119] In some embodiments, an AAV capsid polypeptide or AAV capsid variant described herein may comprise a VOY101 capsid polypeptide, an AAVPHP.B (PHP.B) capsid polypeptide, a AAVPHP.N (PHP.N) capsid polypeptide, an AAV1 capsid polypeptide, an AAV2 capsid polypeptide, an AAV5 capsid polypeptide, an AAV9 capsid polypeptide, an AAV9 K449R capsid polypeptide, an AAVrh10 capsid polypeptide, or a functional variant thereof. In some embodiments, the AAV capsid polypeptide, e.g., AAV capsid variant, comprises an amino acid sequence of any of the AAV capsid polypeptides in Table 6, or an amino acid sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto. In some embodiments, the nucleotide sequence encoding the AAV capsid polypeptide comprises any one of the nucleotide sequences in Table 6, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto.

[0120] In some embodiments, an AAV capsid polypeptide or an AAV capsid variant described herein comprises the amino acid sequence of SEQ ID NO: 138 or an amino acid sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto. In some embodiments the AAV capsid polypeptide or the AAV capsid variant, comprises an amino acid sequence comprising at least one, two, or three modifications,

e.g., substitutions (e.g., conservative substitutions), but no more than 30, 20, or 10 modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of SEQ ID NO: 138. In some embodiments, the AAV capsid polypeptide or the AAV capsid variant, comprises an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 137 or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto. In some embodiments, the nucleotide sequence encoding the AAV capsid polypeptide or the AAV capsid variant comprises the nucleotide sequence of SEQ ID NO: 137 or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto. In some embodiments, the AAV capsid polypeptide or the AAV capsid variant, comprises substitution at position K449, e.g., a K449R substitution, numbered according to SEQ ID NO: 138.

[0121] In some embodiments, the AAV capsid polypeptide or the AAV capsid variant, comprises a peptide comprising the amino acid sequence of TLAVPFK (SEQ ID NO: 4680). In some embodiments, the peptide is present immediately subsequent to position 588, relative to a reference sequence numbered according to SEQ ID NO: 138. In some embodiments, the capsid polypeptide comprises the amino acid substitutions of A587D and Q588G, numbered according to SEQ ID NO: 138.

[0122] In some embodiments, the AAV capsid polypeptide or the AAV capsid variant comprises the amino acid substitution of K449R, numbered according to SEQ ID NO: 138; and a peptide comprising the amino acid sequence of TLAVPFK (SEQ ID NO: 4680), wherein the peptide is present immediately subsequent to position 588, relative to a reference sequence numbered according to SEQ ID NO: 138.

[0123] In some embodiments, the AAV capsid polypeptide or the AAV capsid variant comprises the amino acid substitution of K449R, numbered according to SEQ ID NO: 138; an peptide comprising the amino acid sequence of TLAVPFK (SEQ ID NO: 4680), wherein the insert is present immediately subsequent to position 588, relative to a reference sequence numbered according to SEQ ID NO: 138; and the amino acid substitutions of A587D and Q588G, numbered according to SEQ ID NO: 138.

[0124] In some embodiments, the AAV capsid polypeptide or the AAV capsid variant comprises a peptide comprising the amino acid sequence of TLAVPFK (SEQ ID NO: 4680), wherein the insert is present immediately subsequent to position 588, relative to a reference sequence numbered according to SEQ ID NO: 138; and the amino acid substitutions of A587D and Q588G, numbered according to SEQ ID NO: 138.

[0125] In some embodiments, the AAV capsid polypeptide or the AAV capsid variant comprises the amino acid sequence of SEQ ID NO: 11 or an amino acid sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto.

In some embodiments the AAV capsid polypeptide or the AAV capsid variant, comprises an amino acid sequence comprising at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than 30, 20, or 10 modifications, e.g., substitutions (conservative substitutions), relative to the amino acid sequence of SEQ ID NO: 11, optionally wherein position 449 is not R.

[0126] In some embodiments, the AAV capsid polypeptide or AAV capsid variant, comprises the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto. In some embodiments the AAV capsid polypeptide or the AAV capsid variant, comprises an amino acid sequence comprising at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than 30, 20, or 10 modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of SEQ ID NO: 1.

Table 6. AAV Sequences

Serotype	SEQ ID NO:	Sequence
VOY101	1	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ AKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTE SVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVI TTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQR LINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAH EGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENV PFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIP GPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGS LIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSDGTLAVPFKAQAQT GWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTP VPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEF AVNTEGVYSEPRPIGTRYLTRNL
AAV9/hu.14 K449R	11	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ AKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTE SVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVI TTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQR LINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAH EGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENV PFHSSYAHSQSLDRLMNPLIDQYLYYLSRTINGSGQNQQTLKFSVAGPSNMAVQGRNYIP GPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGS LIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQG ILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPT AFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGV YSEPRPIGTRYLTRNL
AAV9/hu.14 WT (amino acid)	138	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ AKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTE SVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVI TTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQR LINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAH EGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENV PFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIP GPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGS

		LIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQG ILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPT AFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGV YSEPRPIGTRYLTRNL
AAV9/hu.14 WT (DNA)	137	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTTAGTGAAGGAATTCGC GAGTGGTGGGCTTTGAAACCTGGAGCCCCTCAACCCAAGGCAAATCAACAACATCAAGAC AACGCTCGAGGTCTTGTGCTTCCGGGTTACAAATACCTTGGACCCGGCAACGGACTCGAC AAGGGGGAGCCGGTCAACGCAGCAGACGCGGCGGCCCTCGAGCACGACAAGGCCTACGAC CAGCAGCTCAAGGCCGGAGACAACCCGTACCTCAAGTACAACCACGCCGACGCCGAGTTC CAGGAGCGGCTCAAAGAAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAG GCCAAAAAGAGGCTTCTTGAACCTCTTGGTCTGGTTGAGGAAGCGGCTAAGACGGCTCCT GGAAAGAAGAGGCCTGTAGAGCAGTCTCCTCAGGAACCGGACTCCTCCGCGGGTATTGGC AAATCGGGTGCACAGCCCGCTAAAAAGAGACTCAATTTCGGTCAGACTGGCGACACAGAG TCAGTCCCAGACCCTCAACCAATCGGAGAACCTCCCGCAGCCCCCTCAGGTGTGGGATCT CTTACAATGGCTTCAGGTGGTGGCGCACCAGTGGCAGACAATAACGAAGGTGCCGATGGA GTGGGTAGTTCCTCGGGAAATTGGCATTGCGATTCCCAATGGCTGGGGGACAGAGTCATC ACCACCAGCACCCGAACCTGGGCCCTGCCCACCTACAACAATCACCTCTACAAGCAAATC TCCAACAGCACATCTGGAGGATCTTCAAATGACAACGCCTACTTCGGCTACAGCACCCCC TGGGGGTATTTTGACTTCAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCAGCGA CTCATCAACAACAACTGGGGATTCCGGCCTAAGCGACTCAACTTCAAGCTCTTCAACATT CAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCATCGCCAATAACCTTACCAGC ACGGTCCAGGTCTTCACGGACTCAGACTATCAGCTCCCGTACGTGCTCGGGTCGGCTCAC GAGGGCTGCCTCCCGCCGTTCCCAGCGGACGTTTTCATGATTCCTCAGTACGGGTATCTG ACGCTTAATGATGGAAGCCAGGCCGTGGGTCGTTCGTCCTTTTACTGCCTGGAATATTTC CCGTCGCAAATGCTAAGAACGGGTAACAACTTCCAGTTCAGCTACGAGTTTGAGAACGTA CCTTTCCATAGCAGCTACGCTCACAGCCAAAGCCTGGACCGACTAATGAATCCACTCATC GACCAATACTTGTACTATCTCTCAAAGACTATTAACGGTTCTGGACAGAATCAACAAACG CTAAAATTCAGTGTGGCCGGACCCAGCAACATGGCTGTCCAGGGAAGAAACTACATACCT GGACCCAGCTACCGACAACAACGTGTCTCAACCACTGTGACTCAAAACAACAACAGCGAA TTTGCTTGGCCTGGAGCTTCTTCTTGGGCTCTCAATGGACGTAATAGCTTGATGAATCCT GGACCTGCTATGGCCAGCCACAAAGAAGGAGAGGACCGTTTCTTTCCTTTGTCTGGATCT TTAATTTTTGGCAAACAAGGAACTGGAAGAGACAACGTGGATGCGGACAAAGTCATGATA ACCAACGAAGAAGAAATTAAAACTACTAACCCGGTAGCAACGGAGTCCTATGGACAAGTG GCCACAAACCACCAGAGTGCCCAAGCACAGGCGCAGACCGGCTGGGTTCAAAACCAAGGA ATACTTCCGGGTATGGTTTGGCAGGACAGAGATGTGTACCTGCAAGGACCCATTTGGGCC AAAATTCCTCACACGGACGGCAACTTTCACCCTTCTCCGCTGATGGGAGGGTTTGGAATG AAGCACCCGCCTCCTCAGATCCTCATCAAAAACACACCTGTACCTGCGGATCCTCCAACG GCCTTCAACAAGGACAAGCTGAACTCTTTCATCACCCAGTATTCTACTGGCCAAGTCAGC GTGGAGATCGAGTGGGAGCTGCAGAAGGAAAACAGCAAGCGCTGGAACCCGGAGATCCAG TACACTTCCAACTATTACAAGTCTAATAATGTTGAATTTGCTGTTAATACTGAAGGTGTA TATAGTGAACCCCGCCCCATTGGCACCAGATACCTGACTCGTAATCTGTAA

Viral Genome of the AAV particle

[0127] In some embodiments, an AAV particle as described herein comprising an AAV capsid variant described herein, may be used for the delivery of a viral genome to a tissue (e.g., CNS, DRG, and/or muscle). In some embodiments, an AAV particle comprising an AAV capsid variant described herein can be used for delivery of a viral genome to a tissue or cell, e.g., CNS, DRG, or muscle cell or tissue. In some embodiments, an AAV particle of the present disclosure is a recombinant AAV particle. In some embodiments, an AAV particle of the present disclosure is an isolated AAV particle.

[0128] The viral genome may encode any payload, such as but not limited to a polypeptide (e.g., a therapeutic polypeptide), an antibody, an enzyme, an RNAi agent and/or components of a gene editing system. In one embodiment, the AAV particles described herein are used to deliver a payload

to cells of the CNS, after intravenous delivery. In another embodiment, the AAV particles described herein are used to deliver a payload to cells of the DRG, after intravenous delivery. In some embodiments, the AAV particles described herein are used to deliver a payload to cells of a muscle, e.g., a heart muscle, after intravenous delivery.

[0129] In some embodiments, a viral genome of an AAV particle comprising an AAV capsid variant, as described herein, comprises a nucleotide sequence comprising a transgene encoding a payload. In some embodiments, the viral genome comprises an inverted terminal repeat sequence (ITR). In some embodiments, the viral genome comprises two ITR sequences, one at the 5' end of the viral genome (e.g., 5' relative to the encoded payload) and one at the 3' end of the viral genome (e.g., 3' relative to the encoded payload). In some embodiments, a viral genome of an AAV particle, e.g., an AAV particle comprising an AAV capsid variant described herein, may comprise a regulatory element (e.g., promoter), untranslated regions (UTR), a miR binding site, a polyadenylation sequence (polyA), a filler or stuffer sequence, an intron, and/or a linker sequence, e.g., for enhancing transgene expression.

[0130] In some embodiments, the viral genome components are selected and/or engineered for expression of the payload in a target tissue (e.g., CNS, muscle, or DRG).

Viral Genome Component: Inverted Terminal Repeats (ITRs)

[0131] In some embodiments, the AAV particle comprising an AAV capsid variant described herein comprises a viral genome comprising an ITR and a transgene encoding a payload. In some embodiments, the viral genome comprises two ITRs. In some embodiments, the two ITRs flank the nucleotide sequence encoding the payload at the 5' and 3' ends. In some embodiments, the ITRs function as origins of replication comprising recognition sites for replication. In some embodiments, the ITRs comprise sequence regions which can be complementary and symmetrically arranged. In some embodiments, the ITRs incorporated into viral genomes as described herein may be comprised of naturally occurring polynucleotide sequences or recombinantly derived polynucleotide sequences.

[0132] In some embodiments, the ITR may be from the same serotype as the capsid polypeptide, e.g., capsid variant, selected from any of the known serotypes, or a variant thereof. In some embodiments, the ITR may be of a different serotype than the capsid. In some embodiments, the viral genome comprises two ITR sequence regions, wherein the ITRs are of the same serotype as one another. In some embodiments, the viral genome comprises two ITR sequence regions, wherein the ITRs are of different serotypes. Non-limiting examples include zero, one or both of the ITRs having the same serotype as the capsid. In one embodiment both ITRs of the viral genome of the AAV particle are AAV2 ITRs.

[0133] Independently, each ITR may be about 100 to about 150 nucleotides in length. An ITR may be about 100-105 nucleotides in length, 106-110 nucleotides in length, 111-115 nucleotides in length, 116-120 nucleotides in length, 121-125 nucleotides in length, 126-130 nucleotides in length,

131-135 nucleotides in length, 136-140 nucleotides in length, 141-145 nucleotides in length or 146-150 nucleotides in length. In one embodiment, the ITRs are 140-142 nucleotides in length. Non-limiting examples of ITR length are 102, 105, 130, 140, 141, 142, 145 nucleotides in length.

Viral Genome Component: Promoters

[0134] In some embodiments, viral genome of an AAV particle described herein comprises at least one element to enhance the payload target specificity and expression (See e.g., Powell et al. Viral Expression Cassette Elements to Enhance Transgene Target Specificity and Expression in Gene Therapy, 2015; the contents of which are herein incorporated by reference in their entirety). Non-limiting examples of elements to enhance payload target specificity and expression include promoters, endogenous miRNAs, post-transcriptional regulatory elements (PREs), polyadenylation (PolyA) signal sequences and upstream enhancers (USEs), CMV enhancers and introns.

[0135] In some embodiments, an AAV particle comprising an AAV capsid variant described herein comprises a viral genome comprising a nucleic acid comprising a transgene encoding a payload, wherein the transgene is operably linked to a promoter. In some embodiments, the promoter is a species specific promoter, an inducible promoter, a tissue-specific promoter, or a cell cycle-specific promoter (e.g., a promoter as described in Parr et al., *Nat. Med.*3:1145-9 (1997); the contents of which are herein incorporated by reference in their entirety).

[0136] In some embodiments, the Promoter may be naturally occurring or non-naturally occurring. Non-limiting examples of promoters include those derived from viruses, plants, mammals, or humans. In some embodiments, the promoters may be those derived from human cells or systems. In some embodiments, the promoter may be truncated or mutated, e.g., a promoter variant.

[0137] In some embodiments, the promoter is a ubiquitous promoter, e.g., capable of expression in multiple tissues. In some embodiments the promoter is an human elongation factor 1α-subunit (EF1α) promoter, the cytomegalovirus (CMV) immediate-early enhancer and/or promoter, the chicken β-actin (CBA) promoter and its derivative CAG, β glucuronidase (GUSB) promoter, or ubiquitin C (UBC) promoter. In some embodiments, the promoter is a cell or tissue specific promoter, e.g., capable of expression in tissues or cells of the central or peripheral nervous systems, targeted regions within (e.g., frontal cortex), and/or sub-sets of cells therein (e.g., excitatory neurons). In some embodiments, the promoter is a cell-type specific promoters capable of expression of a payload in excitatory neurons (e.g., glutamatergic), inhibitory neurons (e.g., GABA-ergic), neurons of the sympathetic or parasympathetic nervous system, sensory neurons, neurons of the dorsal root ganglia, motor neurons, or supportive cells of the nervous systems such as microglia, glial cells, astrocytes, oligodendrocytes, and/or Schwann cells.

[0138] In some embodiments, the promoter is a liver specific promoter (e.g., hAAT, TBG), skeletal muscle specific promoter (e.g., desmin, MCK, C512), B cell promoter, monocyte promoter, leukocyte promoter, macrophage promoter, pancreatic acinar cell promoter, endothelial cell promoter,

lung tissue promoter, and/or cardiac or cardiovascular promoter (e.g., αMHC, cTnT, and CMV-MLC2k).

[0139] In some embodiments, the promoter is a tissue-specific promoter for payload expression in a tissue or cell of the central nervous system. In some embodiments, the promoter is a synapsin (Syn) promoter, glutamate vesicular transporter (VGLUT) promoter, vesicular GABA transporter (VGAT) promoter, parvalbumin (PV) promoter, sodium channel Na_v 1.8 promoter, tyrosine hydroxylase (TH) promoter, choline acetyltransferase (ChaT) promoter, methyl-CpG binding protein 2 (MeCP2) promoter, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) promoter, metabotropic glutamate receptor 2 (mGluR2) promoter, neurofilament light (NFL) or heavy (NFH) promoter, neuron-specific enolase (NSE) promoter, β-globin minigene nβ2 promoter, preproenkephalin (PPE) promoter, enkephalin (Enk) promoter, and excitatory amino acid transporter 2 (EAAT2) promoter, or a fragment thereof. In some embodiments, the promoter is a cell-type specific promoter capable of expression in an astrocyte, e.g., a glial fibrillary acidic protein (GFAP) promoter and a EAAT2 promoter, or a fragment thereof. In some embodiments, the promoter is a cell-type specific promoter capable of expression in an oligodendrocyte, e.g., a myelin basic protein (MBP) promoter or a fragment thereof.

[0140] In some embodiments, the promoter is a GFAP promoter. In some embodiments, the promoter is a synapsin (syn or syn1) promoter, or a fragment thereof.

[0141] In some embodiments, the promoter comprises an insulin promoter or a fragment thereof.

[0142] In some embodiments, the promoter of the viral genome described herein (e.g., comprised within an AAV particle comprising an AAV capsid variant described herein) comprises an EF-1α promoter or variant thereof, e.g., as provided in Table 8. In some embodiments, the EF-1α promoter comprises the nucleotide sequence of any one of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998-1007 or any one of the sequences provided in Table 8, a nucleotide sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions, relative to the nucleotide sequence of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998-1007 or any one of the sequences provided in Table 8, or a nucleotide sequence with at least 70% (e.g., 80, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998-1007 or any one of the sequences provided in Table 8.

Table 8. Exemplary Promoter Variants

Description	Sequences	SEQ ID NO:
EF1a Promoter (intron underlined)	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGA GAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGG GTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGG GAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTT TGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTT ACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGA TTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGC TTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGC CGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGT CTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGA	987

	TAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCC GCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCC TGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGC TCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTG GCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTG CAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACC CACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACG GAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGT CGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTG GGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTT GCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAA GTTTTTTTCTTCCATTTCAGGTGTCGTGA	
miniEF1a	GCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAG GGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAG TGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATA AGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACAC GCGTAAG	988
Promoter Variant 1	GCATG	
Promoter Variant 2	GGTGGAGAAGAGCATG	990
Promoter Variant 3	GTCATCACTGAGGTGGAGAAGAGCATG	991
Promoter Variant 4	CGTGAG	
Promoter Variant 5	GT	
Promoter Variant 6	GCTCCGGT	
Promoter Variant 19	GCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAG GGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAG TGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATA AGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACAC AG	995
Promoter Variant 20	GCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAG GGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAG TGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATA AGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACAC GC	996
Promoter Variant 7	GTAAG	
Promoter Variant 8	GTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGG AGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAA AGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATA TAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAAC ACGCGTAAG	998
Promoter Variant 9	GCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTT GGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAAC TGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAAC CGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGC CAGAACACGCGTAAG	999
Promoter Variant 10	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGA GAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGG GTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGG GAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTT TGCCGCCAGAACACGCGTAAG	1000
Promoter Variant 11	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGA GAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGG GTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGG	1001

	GAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTT TGCCGCCAGAACACAG	
Promoter Variant 12	GCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTC CCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGC GCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGG TGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAAC GGGTTTGCCGCCAGAACACGCGTAAG	1002
Promoter Variant 13	GCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTC CCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGC GCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGG TGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAAC GGGTTTGCCGCCAGAACACAG	1003
Promoter Variant 14	GGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACAT CGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTA GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTT TTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTC TTTTTCGCAACGGGTTTGCCGCCAGAACACGCGTAAG	1004
Promoter Variant 15	GGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACAT CGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTA GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTT TTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTC TTTTTCGCAACGGGTTTGCCGCCAGAACACAG	1005
Promoter Variant 16	GTCATCACTGAGGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGC AGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGA ACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACT GGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGC CGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACGCGTAAG	1006
Promoter Variant 18	GTCATCACTGAGGTGGAGAAGAGCATGCGTGAGGCTCCGGTGCCCGTCAGTGGGC AGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGA ACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACT GGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGC CGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAG	1007

Viral Genome Component: Untranslated Regions (UTRs)

[0143] In some embodiments, wild type untranslated regions (UTRs) of a gene are transcribed but not translated. Generally, the 5' UTR starts at the transcription start site and ends at the start codon and the 3' UTR starts immediately following the stop codon and continues until the termination signal for transcription.

[0144] Features typically found in abundantly expressed genes of specific target organs (e.g., CNS tissue, muscle, or DRG) may be engineered into UTRs to enhance stability and protein production. As a non-limiting example, a 5' UTR from mRNA normally expressed in the brain (e.g., huntingtin) may be used in the viral genomes of the AAV particles described herein to enhance expression in neuronal cells or other cells of the central nervous system.

[0145] While not wishing to be bound by theory, wild-type 5′ untranslated regions (UTRs) include features which play roles in translation initiation. Kozak sequences, which are commonly known to be involved in the process by which the ribosome initiates translation of many genes, are usually included in 5' UTRs. Kozak sequences have the consensus CCR(A/G)CCAUGG, where R is a purine (adenine or guanine) three bases upstream of the start codon (ATG), which is followed by another 'G'.

[0146] In one embodiment, the 5'UTR in the viral genome includes a Kozak sequence.

[0147] In one embodiment, the 5'UTR in the viral genome does not include a Kozak sequence.

[0148] While not wishing to be bound by theory, wild-type 3′ UTRs are known to have stretches of Adenosines and Uridines embedded therein. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995, the contents of which are herein incorporated by reference in its entirety): Class I AREs, such as, but not limited to, c-Myc and MyoD, contain several dispersed copies of an AUUUA motif within U-rich regions. Class II AREs, such as, but not limited to, GM-CSF and TNF-a, possess two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Class III ARES, such as, but not limited to, c-Jun and Myogenin, are less well defined. These U rich regions do not contain an AUUUA motif. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3′ UTR of nucleic acid molecules will lead to HuR binding and thus, stabilization of the message *in vivo*.

[0149] Introduction, removal or modification of 3′ UTR AU rich elements (AREs) can be used to modulate the stability of a polynucleotide. When engineering specific polynucleotides, e.g., payload regions of viral genomes, one or more copies of an ARE can be introduced to make polynucleotides less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein.

[0150] In one embodiment, the 3' UTR of the viral genome may include an oligo(dT) sequence for templated addition of a poly-A tail.

[0151] In one embodiment, the viral genome may include at least one miRNA seed, binding site or full sequence. microRNAs (or miRNA or miR) are 19-25 nucleotide noncoding RNAs that bind to the sites of nucleic acid targets and down-regulate gene expression either by reducing nucleic acid molecule stability or by inhibiting translation. In some embodiments, a microRNA sequence comprises a seed region, e.g., a sequence in the region of positions 2-8 of the mature microRNA, which has Watson-Crick sequence fully or partially complementarity to the miRNA target sequence of the nucleic acid.

[0152] In one embodiment, the viral genome may be engineered to include, alter or remove at least one miRNA binding site, full sequence or seed region.

[0153] Any UTR from any gene known in the art may be incorporated into the viral genome of the AAV particle. These UTRs, or portions thereof, may be placed in the same orientation as in the gene from which they were selected or they may be altered in orientation or location. In one embodiment, the UTR used in the viral genome of the AAV particle may be inverted, shortened,

lengthened, made with one or more other 5′ UTRs or 3′ UTRs known in the art. As used herein, the term "altered" as it relates to a UTR, means that the UTR has been changed in some way in relation to a reference sequence. For example, a 3′ or 5′ UTR may be altered relative to a wild type or native UTR by the change in orientation or location as taught above or may be altered by the inclusion of additional nucleotides, deletion of nucleotides, swapping or transposition of nucleotides.

[0154] In one embodiment, the viral genome of the AAV particle comprises at least one artificial UTR which is not a variant of a wild type UTR.

[0155] In one embodiment, the viral genome of the AAV particle comprises UTRs which have been selected from a family of transcripts whose proteins share a common function, structure, feature or property.

Viral Genome Component: Polyadenylation Sequence

[0156] The viral genome of the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant, described herein) may comprise a polyadenylation sequence. In some embodiments, the viral genome of the AAV particle (e.g., an AAV particle comprising an AAV capsid variant, described herein) comprises a polyadenylation sequence between the 3' end of the nucleotide sequence encoding the payload and the 5' end of the 3'ITR.

Viral Genome Component: Introns

[0157] In some embodiments, the viral genome of the AAV particle as described herein (e.g., an AAV particle comprising an AAV capsid variant), comprises an element to enhance the payload target specificity and expression (See e.g., Powell et al. *Viral Expression Cassette Elements to Enhance Transgene Target Specificity and Expression in Gene Therapy*, Discov. Med, 2015, 19(102): 49-57; the contents of which are herein incorporated by reference in their entirety), such as an intron. Non-limiting examples of introns include, MVM (67-97 bps), F.IX truncated intron 1 (300 bps), β-globin SD/immunoglobulin heavy chain splice acceptor (250 bps), adenovirus splice donor/immunoglobin splice acceptor (500 bps), SV40 late splice donor/splice acceptor (19S/16S) (180 bps) and hybrid adenovirus splice donor/IgG splice acceptor (230 bps).

Viral Genome Component: Stuffer sequences

[0158] In some embodiments, the viral genome of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant), comprises an element to improve packaging efficiency and expression, such as a stuffer or filler sequence. Non-limiting examples of stuffer sequences include albumin and/or alpha-1 antitrypsin. Any known viral, mammalian, or plant sequence may be manipulated for use as a stuffer sequence.

[0159] In one embodiment, the stuffer or filler sequence may be from about 100-3500 nucleotides in length. The stuffer sequence may have a length of about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900 or 3000 nucleotides.

Viral Genome Component: miRNA

[0160] In one embodiment, the viral genome comprises a sequence encoding a miRNA to reduce the expression of the payload in a tissue or cell, e.g., the DRG (dorsal root ganglion), or neurons of other ganglia, such as those of the sympathetic or parasympathetic nervous system. In some embodiments, a miRNA, e.g., a miR183, a miR182, and/or miR96, may be encoded in the viral genome to modulate, e.g., reduce the expression, of the viral genome in a DRG neuron. As another non-limiting example, a miR-122 miRNA may be encoded in the viral genome to modulate, e.g., reduce, the expression of the viral genome in the liver. In some embodiments, a miRNA, e.g., a miR-142-3p, may be encoded in the viral genome to modulate, e.g., reduce, the expression, of the viral genome in a cell or tissue of the hematopoietic lineage, including for example immune cells (e.g., antigen presenting cells or APC, including dendritic cells (DCs), macrophages, and B-lymphocytes). In some embodiments, a miRNA, e.g., a miR-1, may be encoded in the viral genome to modulate, e.g., reduce, the expression, of the viral genome in a cell or tissue of the heart.

Viral Genome Component: miR Binding Site

[0161] Tissue- or cell-specific expression of the AAV viral particles disclosed herein can be enhanced by introducing tissue- or cell-specific regulatory sequences, e.g., promoters, enhancers, microRNA binding sites, e.g., a detargeting site. Without wishing to be bound by theory, it is believed that an encoded miR binding site can modulate, e.g., prevent, suppress, or otherwise inhibit, the expression of a gene of interest on the viral genome disclosed herein, based on the expression of the corresponding endogenous microRNA (miRNA) or a corresponding controlled exogenous miRNA in a tissue or cell, e.g., a non-targeting cell or tissue. In some embodiments, a miR binding site modulates, e.g., reduces, expression of the payload encoded by a viral genome of an AAV particle described herein in a cell or tissue where the corresponding mRNA is expressed.

[0162] In some embodiments, the viral genome of an AAV particle described herein comprises a nucleotide sequence encoding a microRNA binding site, e.g., a detargeting site. In some embodiments, the viral genome of an AAV particle described herein comprises a nucleotide sequence encoding a miR binding site, a microRNA binding site series (miR BSs), or a reverse complement thereof.

[0163] In some embodiments, the nucleotide sequence encoding the miR binding site series or the miR binding site is located in the 3'-UTR region of the viral genome (e.g., 3' relative to the nucleotide sequence encoding a payload), e.g., before the polyA sequence, 5'-UTR region of the viral genome (e.g., 5' relative to the nucleotide sequence encoding a payload), or both.

[0164] In some embodiments, the encoded miR binding site series comprise at least 1-5 copies, e.g., at least 1-3, 2-4, 3-5, 1, 2, 3, 4, 5 or more copies of a miR binding site (miR BS). In some embodiments, all copies are identical, e.g., comprise the same miR binding site. In some embodiments, the miR binding sites within the encoded miR binding site series are continuous and

not separated by a spacer. In some embodiments, the miR binding sites within an encoded miR binding site series are separated by a spacer, e.g., a non-coding sequence. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides, nucleotides in length. In some embodiments, the spacer coding sequence or reverse complement thereof comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions relative to the nucleotide sequence of GATAGTTA.

[0165] In some embodiments, the encoded miR binding site series comprise at least 1-5 copies, e.g., at least 1-3, 2-4, 3-5, 1, 2, 3, 4, 5 or more copies of a miR binding site (miR BS). In some embodiments, at least 1, 2, 3, 4, 5, or all of the copies are different, e.g., comprise a different miR binding site. In some embodiments, the miR binding sites within the encoded miR binding site series are continuous and not separated by a spacer. In some embodiments, the miR binding sites within an encoded miR binding site series are separated by a spacer, e.g., a non-coding sequence. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides, in length. In some embodiments, the spacer comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions relative to the nucleotide sequence of GATAGTTA.

[0166] In some embodiments, the encoded miR binding site is substantially identical (e.g., at least 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% identical), to the miR in the host cell. In some embodiments, the encoded miR binding site comprises at least 1, 2, 3, 4, or 5 mismatches or no more than 6, 7, 8, 9, or 10 mismatches to a miR in the host cell. In some embodiments, the mismatched nucleotides are contiguous. In some embodiments, the mismatched nucleotides are non-contiguous. In some embodiments, the mismatched nucleotides occur outside the seed region-binding sequence of the miR binding site, such as at one or both ends of the miR binding site. In some embodiments, the miR binding site is 100% identical to the miR in the host cell.

[0167] In some embodiments, the nucleotide sequence encoding the miR binding site is substantially complementary (e.g., at least 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% complementary), to the miR in the host cell. In some embodiments, to complementary sequence of the nucleotide sequence encoding the miR binding site comprises at least 1, 2, 3, 4, or 5 mismatches or no more than 6, 7, 8, 9, or 10 mismatches to a miR in the host cell. In some embodiments, the

mismatched nucleotides are contiguous. In some embodiments, the mismatched nucleotides are non-contiguous. In some embodiments, the mismatched nucleotides occur outside the seed region-binding sequence of the miR binding site, such as at one or both ends of the miR binding site. In some embodiments, the encoded miR binding site is 100% complementary to the miR in the host cell.

[0168] In some embodiments, an encoded miR binding site or sequence region is at least about 10 to about 125 nucleotides in length, e.g., at least about 10 to 50 nucleotides, 10 to 100 nucleotides, 50 to 100 nucleotides, 50 to 125 nucleotides, or 100 to 125 nucleotides in length. In some embodiments, an encoded miR binding site or sequence region is at least about 7 to about 28 nucleotides in length, e.g., at least about 8-28 nucleotides, 7-28 nucleotides, 8-18 nucleotides, 12-28 nucleotides, 20-26 nucleotides, 22 nucleotides, 24 nucleotides, or 26 nucleotides in length, and optionally comprises at least one consecutive region (e.g., 7 or 8 nucleotides) complementary (e.g., fully or partially complementary) to the seed sequence of a miRNA (e.g., a miR122, a miR142, a miR183, or a miR1).

[0169] In some embodiments, the encoded miR binding site is complementary (e.g., fully or partially complementary) to a miR expressed in liver or hepatocytes, such as miR122. In some embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR122 binding site sequence. In some embodiments, the encoded miR122 binding site comprises the nucleotide sequence of ACAAACACCATTGTCACACTCCA (SEQ ID NO: 4673), or a nucleotide sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than ten modifications, e.g., insertions, deletions, or substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of SEQ ID NO: 4673, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 2, 3, 4, or 5 copies of the encoded miR122 binding site, e.g., an encoded miR122 binding site series, optionally wherein the encoded miR122 binding site series comprises the nucleotide sequence of: ACAAACACCATTGTCACACTCCACACAAACACCATTGTCACACTCCACACAAACACCATTGTCACACTCCA (SEQ ID NO: 4674), or a nucleotide sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 4674, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, at least two of the encoded miR122 binding sites are connected directly, e.g., without a spacer. In other embodiments, at least two of the encoded miR122 binding sites are separated by a spacer, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length, which

is located between two or more consecutive encoded miR122 binding site sequences. In embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8, in length. In some embodiments, the spacer coding sequence or reverse complement thereof comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, an encoded miR binding site series comprises at least 3-5 copies (e.g., 4 copies) of a miR122 binding site, with or without a spacer, wherein the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions relative to the nucleotide sequence of GATAGTTA.

[0170] In some embodiments, the encoded miR binding site is complementary (e.g., fully or partially complementary) to a miR expressed in the heart. In embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR-1 binding site. In some embodiments, the encoded miR-1 binding site comprises the nucleotide sequence of ATACATACTTCTTTACATTCCA (SEQ ID NO: 4679), a nucleotide sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 4679, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 2, 3, 4, or 5 copies of the encoded miR-1 binding site, e.g., an encoded miR-1 binding site series. In some embodiments, the at least 2, 3, 4, or 5 copies (e.g., 2 or 3 copies) of the encoded miR-1 binding site are continuous (e.g., not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of GATAGTTA.

[0171] In some embodiments, the encoded miR binding site is complementary (e.g., fully or partially complementary) to a miR expressed in hematopoietic lineage, including immune cells (e.g., antigen presenting cells or APC, including dendritic cells (DCs), macrophages, and B-lymphocytes).

In some embodiments, the encoded miR binding site complementary to a miR expressed in hematopoietic lineage comprises a nucleotide sequence disclosed, e.g., in US 2018/0066279, the contents of which are incorporated by reference herein in its entirety.

[0172] In embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR-142-3p binding site sequence. In some embodiments, the encoded miR-142-3p binding site comprises the nucleotide sequence of TCCATAAAGTAGGAAACACTACA (SEQ ID NO: 4675), a nucleotide sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 4675, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 2, 3, 4, or 5 copies of the encoded miR-142-3p binding site, e.g., an encoded miR-142-3p binding site series. In some embodiments, the at least 2, 3, 4, or 5 copies (e.g., 2 or 3 copies) of the encoded miR-142-3p binding site are continuous (e.g., not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of GATAGTTA.

[0173] In some embodiments, the encoded miR binding site is complementary (e.g., fully complementary or partially complementary) to a miR expressed in a DRG (dorsal root ganglion) neuron, e.g., a miR183, a miR182, and/or miR96 binding site. In some embodiments, the encoded miR binding site is complementary to a miR expressed in expressed in a DRG neuron comprises a nucleotide sequence disclosed, e.g., in WO2020/132455, the contents of which are incorporated by reference herein in its entirety.

[0174] In some embodiments, the encoded miR binding site or encoded miR binding site series comprises a miR183 binding site sequence. In some embodiments, the encoded miR183 binding site comprises the nucleotide sequence of AGTGAATTCTACCAGTGCCATA (SEQ ID NO: 4676), or a nucleotide sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or

deletions, relative to the nucleotide sequence of SEQ ID NO: 4676, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the sequence complementary to the seed sequence corresponds to the double underlined of the encoded miR-183 binding site sequence. In some embodiments, the viral genome comprises at least comprises at least 2, 3, 4, or 5 copies (e.g., at least 2 or 3 copies) of the encoded miR183 binding site, e.g., an encoded miR183 binding site. In some embodiments, the at least 2, 3, 4, or 5 copies (e.g., 2 or 3 copies) of the encoded miR183 binding site are continuous (e.g., not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of GATAGTTA. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii).

[0175] In some embodiments, the encoded miR binding site or the encoded miR binding site series comprises a miR182 binding site sequence. In some embodiments, the encoded miR182 binding site comprises, the nucleotide sequence of AGTGTGAGTTCTACCATTGCCAAA (SEQ ID NO: 4677), a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 4677, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 2, 3, 4, or 5 copies of the encoded miR182 binding site, e.g., an encoded miR182 binding site series. In some embodiments, the at least 2, 3, 4, or 5 copies (e.g., 2 or 3 copies) of the encoded miR182 binding site are continuous (e.g., not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of GATAGTTA. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii).

[0176] In certain embodiments, the encoded miR binding site or the encoded miR binding site series comprises a miR96 binding site sequence. In some embodiments, the encoded miR96 binding site comprises the nucleotide sequence of AGCAAAAATGTGCTAGTGCCAAA (SEQ ID NO: 4678), a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, at least 95%, at least 99%, or 100% sequence identity, or having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of SEQ ID NO: 4678, e.g., wherein the modification can result in a mismatch between the encoded miR binding site and the corresponding miRNA. In some embodiments, the viral genome comprises at least 2, 3, 4, or 5 copies of the encoded miR96 binding site, e.g., an encoded miR96 binding site series. In some embodiments, the at least 2, 3, 4, or 5 copies (e.g., 2 or 3 copies) of the encoded miR96 binding site are continuous (e.g., not separated by a spacer) or separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of GATAGTTA. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii).

[0177] In some embodiments, the encoded miR binding site series comprises a miR122 binding site, a miR-1, a miR142 binding site, a miR183 binding site, a miR182 binding site, a miR 96 binding site, or a combination thereof. In some embodiments, the encoded miR binding site series comprises at least 2, 3, 4, or 5 copies of a miR122 binding site, a miR142 binding site, a miR183 binding site, a miR182 binding site, a miR 96 binding site, or a combination thereof. In some embodiments, at least two of the encoded miR binding sites are connected directly, e.g., without a spacer. In other embodiments, at least two of the encoded miR binding sites are separated by a spacer, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length, which is located between two or more consecutive encoded miR binding site sequences. In embodiments, the spacer is at least about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer coding sequence or reverse complement thereof comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of GATAGTTA.

[0178] In some embodiments, an encoded miR binding site series comprises at least 2-5 copies (e.g., 2 or 3 copies) of a combination of at least two, three, four, five, or all of a miR-1, miR122 binding site, a miR142 binding site, a miR183 binding site, a miR182 binding site, a miR96 binding site, wherein each of the miR binding sites within the series are continuous (e.g., not separated by a spacer) or are separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of GATAGTTA.

[0179] In some embodiments, an encoded miR binding site series comprises at least 2-5 copies (e.g., 2 or 3 copies) of a combination of a miR-122 binding site and a miR-1 binding site, wherein each of the miR binding sites within the series are continuous (e.g., not separated by a spacer) or are separated by a spacer. In some embodiments, the spacer is about 1 to 6 nucleotides or about 5 to 10 nucleotides, e.g., about 7-8 nucleotides or about 8 nucleotides, in length. In some embodiments, the spacer sequence comprises one or more of (i) GGAT; (ii) CACGTG; (iii) GCATGC, or a repeat of one or more of (i)-(iii). In some embodiments, the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), insertions, or deletions, relative to the nucleotide sequence of GATAGTTA.

Genome Size

[0180] In one embodiment, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant), may comprise a single-stranded or double-stranded viral genome. The size of the viral genome may be small, medium, large or the maximum size. As described above, the viral genome may comprise a promoter and a polyA tail.

[0181] In one embodiment, the viral genome may be a small single stranded viral genome. A small single stranded viral genome may be 2.1 to 3.5 kb in size such as, but not limited to, about 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, and 3.5 kb in size.

[0182] In one embodiment, the viral genome may be a small double stranded viral genome. A small double stranded viral genome may be 1.3 to 1.7 kb in size such as, but not limited to, about 1.3, 1.4, 1.5, 1.6, and 1.7 kb in size.

[0183] In one embodiment, the viral genome may be a medium single stranded viral genome. A medium single stranded viral genome may be 3.6 to 4.3 kb in size such as, but not limited to, about 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2 and 4.3 kb in size.

[0184] In one embodiment, the viral genome may be a medium double stranded viral genome. A medium double stranded viral genome may be 1.8 to 2.1 kb in size such as, but not limited to, about 1.8, 1.9, 2.0, and 2.1 kb in size.

[0185] In one embodiment, the viral genome may be a large single stranded viral genome. A large single stranded viral genome may be 4.4 to 6.0 kb in size such as, but not limited to, about 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9 and 6.0 kb in size.

[0186] In one embodiment, the viral genome may be a large double stranded viral genome. A large double stranded viral genome may be 2.2 to 3.0 kb in size such as, but not limited to, about 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 and 3.0 kb in size.

Payloads

[0187] In some embodiments, an AAV particle of the present disclosure (e.g. an AAV particle comprising an AAV capsid variant described herein) comprises a viral genome comprising a nucleic acid encoding a payload. In some embodiments, the encoded payload is an RNAi agent or a polypeptide. A payload of the present disclosure may be, but is not limited to, a peptide, a polypeptide, a protein, an antibody, an RNAi agent, etc.

[0188] In some embodiments, the nucleotide sequence encoding a payload may comprise a combination of coding and non-coding nucleic acid sequences. In some embodiments, the nucleotide sequence encoding the payload may encode a coding or non-coding RNA.

[0189] In some embodiments, the AAV particles described herein, e.g., an AAV particle comprising an AAV capsid variant, comprises a nucleic acid encoding a payload. In some embodiments, the encoded payload comprises a therapeutic protein, an antibody, an enzyme, one or more components of a genome editing system, and/or an RNAi agent (e.g., a dsRNA, siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA). In some embodiments, the encoded payload modulates, e.g., increases or decreases, the presence, level, and/or activity of a gene, mRNA, protein, or a combination thereof, e.g., in a cell or a tissue.

Polypeptides

[0190] In some embodiments, the encoded payload of AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant, described herein comprises a polypeptide, protein, or peptide, e.g., a polypeptide, protein, or peptide described herein. The nucleic acid encoding the payload, may encode a product of any known gene and/or a recombinant version thereof. In some embodiments, the nucleic acid encoding the payload may encode at least one allele of apolipoprotein E (APOE) such as, but not limited to ApoE2, ApoE3 and/or ApoE4. In one embodiment, the nucleic acid encoding the payload encodes ApoE2 (cys112, cys158) protein or a fragment or variant thereof.

In one embodiment, the nucleic acid encoding the payload encodes an ApoE3 (cys112, arg158) protein or fragment or variant thereof. In one embodiment, the nucleic acid encoding the payload encodes ApoE4 (arg112, arg158). As another non-limiting example, the encoded payload comprises an aromatic L-amin acid decarboxylase (AADC) protein. As another non-limiting example, the encoded payload comprises an antibody, or a fragment thereof. As another non-limiting example, the encoded payload comprises a human survival of motor neuron (SMN) 1 or SMN2 protein, or fragments or variants thereof. As another non-limiting example, the encoded payload comprises an aspartoacylase (ASPA) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises a tripeptidyl peptidase I (CLN2) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises a beta-galactosidase (GLB1) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises a N-sulphoglucosamine sulphohydrolase (SGSH) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises an N-acetyl-alpha-glucosaminidase (NAGLU) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises an iduronate 2-sulfatase (IDS) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises an intracellular cholesterol transporter (NPC1) protein, or a fragment or variant thereof. As another non-limiting example, the encoded payload comprises a gigaxonin (GAN) protein, or a fragment or variant thereof. The AAV viral genomes encoding polypeptides described herein may be useful in the fields of human disease, viruses, infections veterinary applications and a variety of *in vivo* and *in vitro* settings.

[0191] Amino acid sequences of a payload polypeptide encoded by a viral genome described herein, may be translated as a whole polypeptide, a plurality of polypeptides or fragments of polypeptides, which independently may be encoded by one or more nucleic acids, fragments of nucleic acids or variants of any of the aforementioned.

Antibodies and Antibody Binding Fragments

[0192] In some embodiments, the encoded payload of AAV particle comprising an AAV capsid variant described herein comprises an antibody or antibody binding fragment. In some embodiments, the antibody may be a full antibody, a fragment, or any functional variant thereof. As non-limiting examples, an antibody may be a native antibody (e.g., with two heavy and two light chains), a heavy chain variable region, a light chain variable region, a heavy chain constant region, a light chain constant region, Fab, Fab', $F(ab')_2$, Fv, or scFv fragments, a diabody, a linear antibody, a single-chain antibody, a multi-specific antibody, an intrabody, one or more heavy chain complementarity determining regions (CDR), one or more light chain CDRs, a bi-specific antibody, a monoclonal antibody, a polyclonal antibody, a humanized antibody, an antibody mimetic, an antibody variant, a miniaturized antibody, a unibody, a maxibody, and/or a chimeric antigen receptor. The encoded antibody or antibody binding fragment may be useful in the treatment of a neurological disease, a

neurodegenerative disorder, a muscular disease, a neuromuscular disorder, a neuro-oncological disorder, or any disorder associated with the central and/or peripheral nervous systems.

[0193] In some embodiments, the viral genome of the AAV particle (e.g., an AAV particle comprising an AAV capsid variant described herein) may comprise a nucleic acid which has been engineered to enable or enhance the expression of an antibody, or antibody binding fragment thereof.

[0194] In some embodiments, the encoded antibody of the payload of an AAV particle comprising an AAV capsid variant, described herein comprises at least one immunoglobulin variable domain sequence. An antibody may include, for example, full-length, mature antibodies and antigen-binding fragments of an antibody. For example, an antibody can include a heavy (H) chain variable domain sequence (VH), and a light (L) chain variable domain sequence (VL). In another example, an antibody includes two heavy (H) chain variable domain sequences and two light (L) chain variable domain sequence, thereby forming two antigen binding sites, such as Fab, Fab', F(ab')2, Fc, Fd, Fd', Fv, single chain antibodies (scFv for example), single variable domain antibodies, diabodies (Dab) (bivalent and bispecific), and chimeric (e.g., humanized) antibodies, which may be produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA technologies. These functional antibody fragments, e.g., an antibody binding fragments, retain the ability to selectively bind with their respective antigen or receptor.

[0195] In some embodiments, the antibody binding fragment comprises at least one portion of an intact antibody, or recombinant variants thereof, and refers to the antigen binding domain, for example, an antigenic determining variable region of an intact antibody, that is sufficient to confer recognition and specific binding of the antibody fragment to a target, such as an antigen. Examples of antigen binding fragments include: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a diabody (dAb) fragment, which consists of a VH domain; (vi) a camelid or camelized variable domain; (vii) a single chain Fv (scFv), see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883); and (viii) a single domain antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. An antibody fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, for example, Hollinger and Hudson, Nature Biotechnology 23:1126-1136, 2005).

[0196] In some embodiments, the encoded antibody of the payload of an AAV particle described herein comprises a multispecific antibody, e.g., it comprises a plurality of immunoglobulin variable domains sequences, wherein a first immunoglobulin variable domain sequence of the plurality has

binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In some embodiments, the first and second epitopes are on the same antigen, e.g., the same protein (or subunit of a multimeric protein). In some embodiments, the first and second epitopes overlap. In some embodiments, the first and second epitopes do not overlap. In some embodiments, the first and second epitopes are on different antigens, e.g., the different proteins (or different subunits of a multimeric protein). In some embodiments, a multispecific antibody comprises a third, fourth or fifth immunoglobulin variable domain. In some embodiments, a multispecific antibody is a bispecific antibody, a trispecific antibody, or tetraspecific antibody.

[0197] In some embodiments, an encoded multispecific antibody of the payload of an AAV particle described herein is an encoded bispecific antibody. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In some embodiments, the first and second epitopes are on the same antigen, e.g., the same protein (or subunit of a multimeric protein). In some embodiments, the first and second epitopes overlap. In some embodiments, the first and second epitopes do not overlap. In some embodiments, the first and second epitopes are on different antigens, e.g., the different proteins (or different subunits of a multimeric protein).

[0198] An antibody or an antibody binding fragment encoded by a viral genome of an AAV particle described herein, may be, but is not limited to, an antibody or antibody fragment that binds to β-amyloid, APOE, tau, SOD1, TDP-43, huntingtin, and/or synuclein. In some embodiments, the encoded payload comprises an antibody or antibody fragment that binds to a neuro-oncology related target, e.g., HER2, EGFR (e.g., EGFRvIII). In some embodiments, the encoded payload comprises an antibody that binds to HER2/neu. In some embodiments, the encoded payload comprises an antibody that binds to β-amyloid. In some embodiments, the encoded payload comprises an antibody that binds to tau.

Gene Editing System

[0199] In some embodiments, the encoded payload of AAV particle comprising an AAV capsid variant described herein comprises a gene editing system or one or more components thereof. In some embodiments, the gene editing system comprises nucleic acid sequences that encode proteins having enzymatic activity to (i) selectively induce double or single stranded breaks in a DNA or RNA sequence, or (ii) substitute, insert or delete a particular base or set of bases of a DNA or RNA sequence in the absence of a double or single stranded break in the DNA or RNA. In some embodiments, the gene editing system includes, but is not limited to a CRISPR-Cas system (including different Cas or Cas-related nucleases), a Zinc finger nuclease, a meganuclease, a TALEN or a base

editors. In some embodiments, the gene editing system comprises a chromosomal integration of a transgene, e.g., introduced by a parvovirus vector in the absence of an exogenous nuclease or an enzymatic entity.

RNAi agents

[0200] In some embodiments, the encoded payload of AAV particle comprising an AAV capsid variant described herein comprises an RNAi agent, e.g., an RNAi agent described herein. In some embodiments, the encoded payload of a viral genome of an AAV particle comprising an AAV capsid variant described herein comprises a dsRNA, a siRNA, a shRNA, a pre-miRNA, a pri-miRNA, a miRNA, a stRNA, a lncRNA, a piRNA, or a snoRNA. In some embodiments, the encoded payload comprises an RNAi agent for inhibiting expression of a SOD1, MAPT, APOE, HTT, TDP-43, APP, BACE, SNCA, ATXN1, ATXN3, ATXN7, SCN1A-SCN5A, or SCN8A-SCN11A gene, protein, and/or mRNA. In some embodiments, the RNAi agent encoded by a viral genome described herein inhibits SOD1, MAPT, APOE, HTT, TDP-43, APP, BACE, SNCA, ATXN1, ATXN3, ATXN7, SCN1A-SCN5A, or SCN8A-SCN11A.

[0201] An AAV particle comprising an AAV capsid variant described herein may comprise a viral genome encoding an RNAi agent, which targets the mRNA of a gene to modulate, e.g., interfere with gene expression and/or protein production.

[0202] In some embodiments, the RNAi agent may target a gene at the location of a single-nucleotide polymorphism (SNP) or variant within the nucleotide sequence of the gene.

[0203] The RNAi agent may be an siRNA duplex, wherein the siRNA duplex contains an antisense strand (guide strand) and a sense strand (passenger strand) hybridized together forming a duplex structure, wherein the antisense strand is complementary to the nucleic acid sequence of the targeted gene, and wherein the sense strand is homologous to the nucleic acid sequence of the targeted gene. In some aspects, the 5'end of the antisense strand has a 5' phosphate group and the 3'end of the sense strand contains a 3'hydroxyl group. In other aspects, there are none, one or 2 nucleotide overhangs at the 3'end of each strand.

[0204] Each strand of an siRNA duplex targeting a gene of interest may be about 19 to 25, 19 to 24 or 19 to 21 nucleotides in length, preferably about 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, or 25 nucleotides in length.

[0205] In one embodiment, an siRNA or dsRNA includes at least two sequences that are complementary to each other. The dsRNA includes a sense strand having a first sequence and an antisense strand having a second sequence. The antisense strand includes a nucleotide sequence that is substantially complementary to at least part of an mRNA encoding the target gene, and the region of complementarity is 30 nucleotides or less, and at least 15 nucleotides in length. Generally, the dsRNA is 19 to 25, 19 to 24 or 19 to 21 nucleotides in length. In some embodiments, the dsRNA is from about 15 to about 25 nucleotides in length, and in other embodiments the dsRNA is from about

25 to about 30 nucleotides in length. In some embodiments, the dsRNA is about 15 nucleotides in length, 16 nucleotides in length, 17 nucleotides in length, 18 nucleotides in length, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides in length, 26 nucleotides in length, 27 nucleotides in length, 28 nucleotides in length, 29 nucleotides in length, or 30 nucleotides in length.

[0206] In some embodiments, the encoded RNAi agent is a siRNA.

[0207] In some embodiments, the RNAi agent, e.g., an RNAi agent described herein inhibits the expression of the gene, mRNA, and/or protein by at least 10%, at least 20%, at least 25%, at least 30%, at least 35% or at least 40% or more, such as when assayed by a method known in the art. In some embodiments, the RNAi agent inhibits expression of a gene, mRNA, and protein by 50-100%, e.g., by 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%.

[0208] In some embodiments, the AAV particle described herein, comprising a viral genome encoding an RNAi agent targeting a gene of interest is administered to a subject in need for treating and/or ameliorating a disease, e.g., a neurological disorder of any disease associated with the central or peripheral nervous systems.

Design of siRNA

[0209] An AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) may comprise a viral genome encoding a siRNA molecule (e.g., siRNA duplex or encoded dsRNA) that target a gene of interest and suppress target gene expression, mRNA expression, and protein production. In some aspects, the siRNA molecules are designed and used to knock out target gene variants in cells, e.g., transcripts that are identified in neurological disease. In some aspects, the siRNA molecules are designed and used to knock down target gene variants in cells.

[0210] Some guidelines for designing siRNAs (for insertion into a viral genome of the AAV particles described herein) have been proposed in the art. These guidelines generally recommend generating a 19-nucleotide duplexed region, symmetric 2-3 nucleotide 3'overhangs, 5-phosphate and 3-hydroxyl groups targeting a region in the gene to be silenced. Other rules that may govern siRNA sequence preference include, but are not limited to, (i) A/U at the 5′ end of the antisense strand; (ii) G/C at the 5′ end of the sense strand; (iii) at least five A/U residues in the 5′ terminal one-third of the antisense strand; and (iv) the absence of any GC stretch of more than 9 nucleotides in length. In accordance with such considerations, together with the specific sequence of a target gene, highly effective siRNA molecules essential for suppressing mammalian target gene expression may be readily designed.

[0211] In one embodiment, the sense and/or antisense strand is designed based on the method and rules outlined in European Patent Publication No. EP1752536, the contents of which are herein incorporated by reference in their entirety. As a non-limiting example, the 3'-terminal base of the sequence is adenine, thymine or uracil. As a non-limiting example, the 5'-terminal base of the

sequence is guanine or cytosine. As a non-limiting example, the 3'-terminal sequence comprises seven bases rich in one or more bases of adenine, thymine and uracil.

[0212] In one embodiment, an siRNA molecule comprises a sense strand and a complementary antisense strand in which both strands are hybridized together to form a duplex structure. The antisense strand has sufficient complementarity to the target mRNA sequence to direct target-specific RNAi, e.g., the siRNA molecule has a sequence sufficient to trigger the destruction of the target mRNA by the RNAi machinery or process.

[0213] In some embodiments, the antisense strand and target mRNA sequences have 100% complementarity. The antisense strand may be complementary to any part of the target mRNA sequence. Neither the identity of the sense sequence nor the homology of the antisense sequence need be 100% complementary to the target.

[0214] In other embodiments, the antisense strand and target mRNA sequences comprise at least one mismatch. As a non-limiting example, the antisense strand and the target mRNA sequence have at least 50-90%, 50-95%, 50-99%, 60-70%, 60-80%, 60-90%, 60-95%, 60-99%, 70-80%, 70-90%, 70-95%, 70-99%, 80-90%, 80-95%, 80-99%, 90-95%, 90-99% or 95-99% complementary.

[0215] The siRNA molecule may have a length from about 10-50 or more nucleotides, e.g., each strand comprising 10-50 nucleotides (or nucleotide analogs). Preferably, the siRNA molecule has a length from about 15-30, e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in each strand, wherein one of the strands is sufficiently complementary to a target region. In one embodiment, the siRNA molecule has a length from about 19 to 25, 19 to 24 or 19 to 21 nucleotides.

[0216] In some embodiments, the siRNA molecule can be a synthetic RNA duplex comprising about 19 nucleotides to about 25 nucleotides, and two overhanging nucleotides at the 3'-end.

[0217] The siRNA molecule may comprise an antisense sequence and a sense sequence, or a fragment or variant thereof. As a non-limiting example, the antisense sequence and the sense sequence have at least 50-90%, 50-95%, 50-99%, 60-70%, 60-80%, 60-90%, 60-95%, 60-99%, 70-80%, 70-90%, 70-95%, 70-99%, 80-90%, 80-95%, 80-99%, 90-95%, 90-99% or 95-99% complementary.

[0218] The sense and antisense sequences may be completely complementary across a substantial portion of their length. In other embodiments, the sense sequence and antisense sequence may be at least 70, 80, 90, 95 or 99% complementary across independently at least 50, 60, 70, 80, 85, 90, 95, or 99% of the length of the strands.

[0219] In some embodiments, the sense and antisense strands of a siRNA duplex are linked by a short spacer sequence leading to the expression of a stem-loop structure termed short hairpin RNA (shRNA). The hairpin is recognized and cleaved by Dicer, thus generating mature siRNA molecules.

[0220] In some embodiments, the siRNA molecules, as well as associated spacer and/or flanking regions once designed, can be encoded by the viral genome of the AAV particles described herein, for delivery to a cell.

Molecular Scaffold

[0221] In some embodiments, the siRNA molecules may be encoded in a modulatory polynucleotide which also comprises a molecular scaffold.

[0222] In some embodiments, the modulatory polynucleotide which comprises the payload (e.g., siRNA, miRNA or other RNAi agent described herein) includes a molecular scaffold which comprises a 5' flanking sequence, a loop region, and/or a 3' flanking region. In some embodiments a 5' or 3' flanking region may be of any length and may a wild type microRNA sequence or a portion thereof, or may be completely artificial. A 3' flanking sequence may mirror the 5' flanking sequence in size and origin. Either flanking sequence may be absent. In one embodiment, both the 5' and 3' flanking sequences are absent. The 3' flanking sequence may optionally contain one or more CNNC motifs, where "N" represents any nucleotide. In some embodiments, the loop comprises at least one UGUG motif. In some embodiments, the UGUG motif is located at the 5' terminus of the loop. In some embodiments the 5' and 3' flanking sequences are the same sequence. In some embodiments they differ by 2%, 3%, 4%, 5%, 10%, 20% or more than 30% when aligned to each other.

[0223] In some embodiments, modulatory polynucleotide comprises a stem loop structure. In some embodiments, the modulatory polynucleotide comprises in 5' to 3' order: a 5' flanking sequence, a guide strand sequence, a loop region, a passenger strand sequence, and a 3' flanking sequence. In some embodiments, the modulatory polynucleotide comprises in 5' to 3' order: a 5' flanking sequence, a passenger strand sequence, a loop region, a guide strand sequence, and a 3' flanking sequence.

[0224] In one embodiment, the molecular scaffold comprises a dual-function targeting modulatory polynucleotide.

[0225] In one embodiment, the molecular scaffold may comprise one or more linkers known in the art. The linkers may separate regions or one molecular scaffold from another. As a non-limiting example, the molecular scaffold may be polycistronic.

[0226] In one embodiment, the modulatory polynucleotide is designed using at least one of the following properties: loop variant, seed mismatch/bulge/wobble variant, stem mismatch, loop variant and basal stem mismatch variant, seed mismatch and basal stem mismatch variant, stem mismatch and basal stem mismatch variant, seed wobble and basal stem wobble variant, or a stem sequence variant.

AAV production

[0227] Viral production disclosed herein describes processes and methods for producing AAV particles (with enhanced, improved and/or increased tropism for a target tissue), e.g., an AAV particle comprising an AAV capsid variant that may be used to contact a target cell to deliver a payload.

[0228] In some embodiments, disclosed herein is a method of making AAV particle of the present disclosure, e.g., an AAV particle comprising an AAV capsid variant the method comprising: (i) providing a host cell comprising a viral genome described herein and (ii) incubating the host cell under conditions suitable to enclose the viral genome in an AAV capsid variant, e.g., an AAV capsid variant described herein (e.g., an AAV capsid variant listed in Table 4), thereby making the AAV particle. In some embodiments, the method comprises prior to step (i), introducing a first nucleic acid comprising the viral genome into a cell. In some embodiments, the host cell comprises a second nucleic acid encoding the AAV capsid variant. In some embodiments, the second nucleic acid is introduced into the host cell prior to, concurrently with, or after the first nucleic acid molecule. In some embodiments, the AAV particle described herein is an isolated AAV particle. In some embodiments, the AAV particle described herein is a recombinant AAV particle.

[0229] Any method known in the art may be used for the preparation of AAV particles. In some embodiments, AAV particles are produced in mammalian cells (e.g., HEK293). In another embodiment, AAV particles are produced in insect cells (e.g., Sf9).

[0230] Methods of making AAV particles are well known in the art and are described in e.g., U.S. Patent Nos. US6204059, US5756283, US6258595, US6261551, US6270996, US6281010, US6365394, US6475769, US6482634, US6485966, US6943019, US6953690, US7022519, US7238526, US7291498 and US7491508, US5064764, US6194191, US6566118, US8137948; or International Publication Nos. WO1996039530, WO1998010088, WO1999014354, WO1999015685, WO1999047691, WO2000055342, WO2000075353 and WO2001023597; Methods In Molecular Biology, ed. Richard, Humana Press, NJ (1995); O'Reilly et al., Baculovirus Expression Vectors, A Laboratory Manual, Oxford Univ. Press (1994); Samulski et al., *J. Vir.*63:3822-8 (1989); Kajigaya et al., *Proc. Nat'l. Acad. Sci. USA* 88: 4646-50 (1991); Ruffing et al., *J. Vir.* 66:6922-30 (1992); Kimbauer et al., *Vir.*, 219:37-44 (1996); Zhao et al., *Vir.*272:382-93 (2000); the contents of each of which are herein incorporated by reference in their entirety. In some embodiments, the AAV particles are made using the methods described in International Patent Publication WO2015191508, the contents of which are herein incorporated by reference in their entirety.

Therapeutic Applications

[0231] The present disclosure provides a method for treating a disease, disorder and/or condition in a subject, including a human subject, comprising administering to the subject an AAV particle described herein, e.g., an AAV particle comprising an AAV capsid variant (e.g., an AAV capsid variant described herein), or administering to the subject any of the described compositions, including a pharmaceutical composition, described herein.

[0232] In some embodiments, the AAV particle (e.g., an AAV particle comprising an AAV capsid variant) is administered to a subject prophylactically, to prevent on-set of disease. In another embodiment, the AAV particle (e.g., an AAV particle comprising an AAV capsid variant) is

administered to treat (e.g., lessen the effects of) a disease or symptoms thereof. In yet another embodiment, the AAV particle (e.g., an AAV particle comprising an AAV capsid variant) is administered to cure (eliminate) a disease. In another embodiment, the AAV particle (e.g., an AAV particle comprising an AAV capsid variant) of the present disclosure is administered to prevent or slow progression of disease. In yet another embodiment, the AAV particle (e.g., an AAV particle comprising an AAV capsid variant) of the present disclosure are used to reverse the deleterious effects of a disease. Disease status and/or progression may be determined or monitored by standard methods known in the art.

[0233] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for treatment, prophylaxis, palliation or amelioration of a genetic disorder, e.g., an autosomal dominant genetic disorder, an autosomal recessive disorder, X-linked dominant genetic disorder, an X-linked recessive genetic disorder, or a Y-linked genetic disorder. In some embodiments, the genetic disorder is a monogenetic disorder or a polygenic disorder. In some embodiments, treatment of a genetic disorder, e.g., a monogenic disorder, comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy.

[0234] In some embodiments, provided herein is method for treating a neurological disorder and/or neurodegenerative disorder in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition described herein or an AAV particle, e.g., a plurality of particles, comprising an AAV capsid variant described herein. In some embodiments, treatment of a neurological disorder and/or neurodegenerative disorder comprises prevention of said neurological disorder and/or neurological disorder.

[0235] In some embodiments, the AAV particle (e.g., an AAV particle comprising an AAV capsid variant) of the disclosure is useful for the treatment, prophylaxis, palliation or amelioration of neurological diseases and/or disorders. In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of tauopathy.

[0236] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is for the treatment, prophylaxis, palliation or amelioration of Alzheimer's Disease. In some embodiments, treatment of Alzheimer's Disease comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an ApoE2 protein, ApoE4 protein, an ApoE3 protein, BDNF protein, CYP46A1 protein, Klotho protein, fractalkine (FKN) protein, neprilysin protein (NEP), CD74 protein, caveolin-1, or a combination or variant thereof. In some embodiments, treatment of Alzheimer's Disease comprises the use of an AAV particle described

herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a reduction in the expression of a tau gene and/or protein, a synuclein gene and/or protein, or a combination or variant thereof. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an antibody that binds to tau or synuclein, an RNAi agent for inhibiting tau or synuclein, a gene editing system (e.g., a CRISPR-Cas system) for altering tau or synuclein expression, or a combination thereof.

[0237] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful the treatment, prophylaxis, palliation or amelioration of Friedreich's ataxia, or any disease stemming from a loss or partial loss of frataxin protein.

[0238] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is for the treatment, prophylaxis, palliation or amelioration of frontal temporal dementia. In some embodiments, treatment of frontal temporal dementia comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy.

[0239] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of Parkinson's Disease. In some embodiments, treatment of Parkinson's disease comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an AADC protein, GAD protein, GDNF protein, TH-GCH1 protein, AIMP2-DX2 protein, or a combination or variant thereof. In some embodiments, treatment of Parkinson's disease comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene knock-down therapy or a gene editing therapy (e.g., knock-out, repression, or correction). In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a modulator, e.g., an RNAi agent or a CRISPR-Cas system, for altering expression of an alpha-synuclein gene, mRNA, and/or protein, or variant thereof. In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of an AADC deficiency. In some embodiments, treatment of AADC deficiency comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an AADC protein or variant thereof.

[0240] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of Amyotrophic lateral sclerosis. In some embodiments, treatment of ALS comprises the use of an

AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a TDP-43 protein, UPF1 protein, CCNF protein, HSF1 protein, Factor H protein, NGF protein, ADAR2 protein, GDNF protein, VEGF protein, HGF protein, NRTN protein, AIMP2-DX2 protein, or a combination or variant thereof. In some embodiments, treatment of ALS comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene knock-down therapy or a gene editing therapy (e.g., knock-out, repression, or correction). In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a modulator, e.g., an RNAi agent or a CRISPR-Cas system, for altering expression of a SOD1 gene, mRNA, and/or protein, or a combination or variant thereof.

[0241] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of Huntington's Disease. In some embodiments, treatment of ALS comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene knock-down (e.g., knock-out) therapy or a gene editing therapy (e.g., knock-out, repression, or correction). In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a modulator, e.g., an RNAi agent or a CRISPR-Cas system, for altering expression of an HTT gene, mRNA, and/or protein, or a variant thereof.

[0242] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of spinal muscular atrophy. In some embodiments, treatment of spinal muscular atrophy comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an SMN1 protein, an SMN2 protein, or a combination or variant thereof.

[0243] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of multiple system atrophy. In some embodiments, treatment of multiple system atrophy comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy.

[0244] n some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of Gaucher disease (GD) (e.g., Type 1 GD, Type 2 GD, or Type 3 GD). In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for

the treatment, prophylaxis, palliation or amelioration of Parkinson's disease. In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of dementia with Lewy Bodies (DLB).

[0245] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for treatment, prophylaxis, palliation or amelioration of a leukodystrophy, e.g., Alexander disease, autosomal dominant leukodystrophy with autonomic diseases (ADLD), Canavan disease, cerebrotendinous xanthomatosis (CTX), metachromatic leukodystrophy (MLD), Pelizaeus-Merzbacher disease, or Refsum disease. In some embodiments, treatment of MLD comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an ARSA protein or variant thereof. In some embodiments, treatment of ALD comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an ABCD-1 protein or variant thereof.

[0246] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of megalencephalic leukoencephalopathy (MLC). In some embodiments, treatment of MLC comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an MLC1 protein or variant thereof.

[0247] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Krabbe disease. In some embodiments, treatment of Krabbe disease comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy.

[0248] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Mucopolysaccharidosis, e.g., a Type I (MPS I), Type II (MPS II), Type IIIA (MPS IIIA), Type IIIB (MPS IIIB), or Type IIIC (MPS IIIC). In some embodiments, treatment of Mucopolysaccharidosis comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy or a gene editing therapy (e.g., enhancement or correction). In some embodiments, the payload encoded or corrected by an AAV

particle comprising a capsid variant described herein comprises an IDUA protein, IDS protein, SGSH protein, NAGLU protein, HGSNAT protein, or a combination or variant thereof.

[0249] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Batten/NCL. In some embodiments, treatment of Batten/NCL comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a CLN1 protein, CLN2 protein, CLN3 protein, CLN5 protein, CLN6 protein, CLN7 protein, CLN8 protein, or a combination or variant thereof.

[0250] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of Rett Syndrome. In some embodiments, treatment of Rett Syndrome comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an MeCP2 protein or variant thereof.

[0251] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Angelman Syndrome. In some embodiments, treatment of Angelman Syndrome comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a UBE3A protein or variant thereof.

[0252] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Fragile X Syndrome. In some embodiments, treatment of Fragile X Syndrome comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a Reelin protein, a DgkK protein, a FMR1 protein, or a combination or variant thereof.

[0253] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Canavan Disease. In some embodiments, treatment of Canavan Disease comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an ASPA protein or variant thereof.

[0254] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Gangliosidosis, e.g., a GM1 Gangliosidosis or a GM2 Gangliosidosis (e.g., Tay Sachs Sandhoff). In some embodiments, treatment of a Gangliosidosis, e.g., a GM1 Gangliosidosis or a GM2 Gangliosidosis (e.g., Tay Sachs Sandhoff), comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a GLB1 protein, a HEXA protein, a HEXB protein, a GM2A protein, or a combination or variant thereof.

[0255] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of GM3 Synthase Deficiency. In some embodiments, treatment of GM3 Synthase Deficiency comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an ST3GAL5 protein or variant thereof.

[0256] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Niemann-Pick disorder, e.g., a Niemann-Pick A or a Niemann-Pick C1 (NPC-1). In some embodiments, treatment of a Niemann-Pick disorder, e.g., a Niemann-Pick A or a Niemann-Pick C1 (NPC-1) comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an ASM protein, an NPC1 protein, or variant thereof.

[0257] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Schwannoma (e.g., Neuroma). In some embodiments, treatment of Schwannoma (e.g., Neuroma) comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a Caspase-1 protein or variant thereof.

[0258] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Tuberous Sclerosis, e.g., Tuberous Sclerosis Type 1 or Tuberous Sclerosis Type 2. In some embodiments, treatment of Tuberous Sclerosis, e.g., Tuberous Sclerosis Type 1 or Tuberous Sclerosis Type 2 comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an

AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a TSC1 protein, a TSC2 protein, or variant thereof.

[0259]

[0260] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Charcot-Marie-Tooth disorder, e.g., a Charcot-Marie-Tooth Type 1X (CMT1X) disorder, a Charcot-Marie-Tooth Type 2A (CMT2A) disorder, or a Charcot-Marie-Tooth Type 4J (CMT4J) disorder. In some embodiments, treatment of a Charcot-Marie-Tooth disorder, e.g., a Charcot-Marie-Tooth Type 1X (CMT1X) disorder, a Charcot-Marie-Tooth Type 2A (CMT2A) disorder, or a Charcot-Marie-Tooth Type 4J (CMT4J) disorder, comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a GJB1 protein, a MFN2 protein, a FIG4 protein, or variant thereof.

[0261] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of an Aspartylglucosaminuria (AGU). In some embodiments, treatment of an AGU comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an AGA protein or variant thereof.

[0262] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Leigh Syndrome. In some embodiments, treatment of a Leigh Syndrome comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a SURF1 protein or variant thereof.

[0263] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of epilepsy. In some embodiments, treatment of epilepsy comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an NPY/Y2 protein, a Galanin protein, a Dynorphin protein, an AIMP2-DX2 protein, an SLC6A1 protein, an SLC13A5 protein, a KCNQ2 protein, or variant thereof.

[0264] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Dravet Syndrome. In some embodiments, treatment of Dravet Syndrome comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises an SCN1a protein, or variant thereof.

[0265] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of a Duchenne muscular dystrophy (DMD). In some embodiments, treatment of DMD comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy or enhancement (e.g., correction of exon-skipping), or a gene editing therapy (e.g., enhancement or correction). In some embodiments, the payload encoded or corrected by an AAV particle comprising a capsid variant described herein comprises a Dystrophin gene and/or protein, a Utrophin gene and/or protein, or a GALGT2 gene and/or protein, or a Follistatin gene and/or protein, or a combination or variant thereof.

[0266] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Pompe Disease. In some embodiments, treatment of Pompe Disease comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a GAA protein, or variant thereof.

[0267] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation, or amelioration of Limb-Girdle Muscular Dystrophy (LGMD2A). In some embodiments, treatment of LGMD2A comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy. In some embodiments, the payload encoded by an AAV particle comprising a capsid variant described herein comprises a CAPN-3 protein, DYSF protein, a SGCG protein, a SGCA protein, a SGCB protein, a FKRP protein, a ANO5 protein, or a combination or variant thereof.

[0268] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of chronic or neuropathic pain.

[0269] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising AAV capsid variant) is useful for treatment, prophylaxis, palliation or amelioration of a disease associated with the central nervous system.

[0270] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for treatment, prophylaxis, palliation or amelioration of a disease associated with the peripheral nervous system.

[0271] In some embodiments, provided herein is a method for treating a neuro-oncological disorder in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition described herein or an AAV particle, e.g., a plurality of particles, comprising an AAV capsid variant described herein. In some embodiments, treatment of a neuro-oncological disorder comprises prevention of said neuro-oncological disorder. In some embodiments, a neuro-oncological disorder comprises a cancer of a primary CNS origin (e.g., a CNS cell, a tissue, or a region), or a metastatic cancer in a CNS cell, tissue, or region. Examples of primary CNS cancers could be gliomas (which may include glioblastoma (also known as glioblastoma multiforme), astrocytomas, oligodendrogliomas, and ependymomas, and mixed gliomas), meningiomas, medulloblastomas, neuromas, and primary CNS lymphoma (in the brain, spinal cord, or meninges), among others. Examples of metastatic cancers include those originating in another tissue or organ, e.g., breast, lung, lymphoma, leukemia, melanoma (skin cancer), colon, kidney, prostate, or other types that metastasize to brain.

[0272] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of a disease associated with expression of HER2, e.g., a disease associated with overexpression of HER2. In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of a HER2-positive cancer. In some embodiments, the HER2-positive cancer is a HER2-positive solid tumor. Additionally, or alternatively, the HER2-positive cancer may be a locally advanced or metastatic HER2-positive cancer. In some instances, the HER2-positive cancer is a HER2-positive breast cancer or a HER2-positive gastric cancer. In some embodiments, the HER2-positive cancer is selected from the group consisting of a HER2- positive gastroesophageal junction cancer, a HER2-positive colorectal cancer, a HER2-positive lung cancer (e.g., a HER2-positive non-small cell lung carcinoma), a HER2-positive pancreatic cancer, a HER2-positive colorectal cancer, a HER2-positive bladder cancer, a HER2-positive salivary duct cancer, a HER2-positive ovarian cancer (e.g., a HER2-positive epithelial ovarian cancer), or a HER2-positive endometrial cancer. In some instances, the HER2-positive cancer is prostate cancer. In some embodiments, the HER2-positive cancer has metastasized to the central nervous system (CNS). In some instances, the metastasized HER2-cancer has formed CNS neoplasms.

[0273] In some embodiments, the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) is administered to a subject having at least one of the diseases or symptoms described herein. In some embodiments, an AAV

particle of the present disclosure is administered to a subject having or diagnosed with having a disease or disorder described herein.

[0274] In some embodiments, provided herein is a method for treating a muscular disorder and/or neuromuscular disorder in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition described herein or an AAV particle, e.g., a plurality of particles, comprising an AAV capsid variant described herein. In some embodiments, treatment of a muscular disorder and/or neuromuscular disorder comprises prevention of said muscular disorder and/or neuromuscular disorder.

[0275] In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for treatment, prophylaxis, palliation or amelioration of a cardiac disease or heart disease and/or method of improving (e.g., enhancing) cardiac function in a subject. In some embodiments, the cardiac disease is a cardiomyopathy (e.g., arrhythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy, or hypertrophic cardiomyopathy), congestive heart failure, tachycardia (e.g., catecholaminergic polymorphic ventricular tachycardia), ischemic heart disease, and/or myocardial infarction. In some embodiments, the cardiac disease is a disease associated with expression, e.g., aberrant expression, of LAMP2B, MYBPC3, TNNI3, LMNA, BAG3, DWORF, PKP2, Cx43, TAZ, CASQ2, SERCA2a, I-1c, S100A1 and/or ARC, S100A1, ASCL1, miR133, Mydelta3, Sav, or a combination or variant thereof. In some embodiments, treatment of a cardiac disorder described herein comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy.

[0276] In some embodiments, the cardiac disease is a genetic disorder, e.g., an autosomal dominant genetic disorder, an autosomal recessive disorder, or an X-linked recessive genetic disorder. In some embodiments, the cardiomyopathy is a genetic disorder, e.g., a genetic disorder associated with an abnormality (e.g., mutation, insertion, rearrangement and/or deletion) in a gene chosen from TTN, LMNA, MYH7, MYH6, SCN5A, TNNT2, RBM20, TNNI3, MYL2, MYL3, PKP2, DSP, DSG2, DSC2, JUP, or a combination thereof. In some embodiments, the cardiac disorder is a dilated cardiomyopathy, e.g., a dilated cardiomyopathy associated with an abnormality (e.g., mutation, insertion, rearrangement and/or deletion) in a gene chosen from TTN, LMNA, MIH7, BAG3, MIPN, TNNT2, SCN5A, RBN20, TNPO, LAMA4, VCL, LDB3, TCAP, PSEN1/2, ACTN2, CRYAB, TPM1, ABCC9, ACTC1, PDLIM3, ILK, TNNC1, TNNI3, PLN, DES, SGCD, CSRP3, MIH6, EYA4, ANKRD1, DMD, GATAD1, TAZ/G4.5, or combination thereof. In some embodiments, the cardiac disorder is a hypertrophic cardiomyopathy, e.g., a hypertrophic cardiomyopathy associated with an abnormality (e.g., mutation, insertion, rearrangement and/or deletion) in a gene chosen from MYH7, TNNT2, TNNI3, TPM1, MYL2, MYL3, ACTC1, CSRP3, TTN, ACTN2, MYH6, TCAP, TNNC1, or a combination thereof. In some embodiments, the cardiac disorder is an arrhythmogenic ventricular

cardiomyopathy, e.g., an arrhythmogenic ventricular cardiomyopathy associated with an abnormality (e.g., mutation, insertion, rearrangement and/or deletion) in a gene chosen from PKP2, DSG2, DSP, RYR2, DSC2, TGFB3, TMEM43, DES, TTN, LMNA, or a combination thereof.

[0277] In some embodiments, the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) is administered to a subject having at least one of the diseases or symptoms described herein. In some embodiments, an AAV particle of the present disclosure is administered to a subject having or diagnosed with having a disease or disorder described herein.

[0278] Any neurological disease or disorder, neurodegenerative disorder, muscular disorder, neuromuscular disorder, and/or neuro-oncological disorder may be treated with the AAV particles of the disclosure, or pharmaceutical compositions thereof.

Pharmaceutical Composition and Formulations

[0279] According to the present disclosure, an AAV particle comprising an AAV capsid variant described herein may be prepared as a pharmaceutical composition. In some embodiments, the pharmaceutical composition comprises at least one active ingredients. In some embodiments, the pharmaceutical composition comprises a pharmaceutically acceptable excipient.

[0280] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) can be formulated using an excipient to: (1) increase stability; (2) increase cell transfection or transduction; (3) permit the sustained or delayed expression of the payload; (4) alter the biodistribution (e.g., target the viral particle to specific tissues or cell types); (5) increase the translation of encoded protein; (6) alter the release profile of encoded protein; and/or (7) allow for regulatable expression of the payload. Formulations of the present disclosure can include, without limitation, saline, liposomes, lipid nanoparticles, polymers, peptides, proteins, cells transfected with viral vectors (e.g., for transfer or transplantation into a subject) and combinations thereof.

[0281] In some embodiments, the relative amount of the active ingredient (e.g. an AAV particle comprising an AAV capsid variant described herein), a pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the present disclosure may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the composition is to be administered. For example, the composition may comprise between 0.1% and 99% (w/w) of the active ingredient. By way of example, the composition may comprise between 0.1% and 100%, e.g., between .5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

[0282] In some embodiments, the pharmaceutical composition comprising an AAV particle described herein may comprise an AAV capsid variant and a viral genome encoding a payload, e.g., a payload described herein, with or without a pharmaceutically acceptable excipient.

[0283] The present disclosure also provides in some embodiments, a pharmaceutical composition suitable for administration to a subject, e.g., a human. In some embodiments, the pharmaceutical composition is administered to a subject, e.g., a human.

Administration

[0284] In some embodiments, an AAV particle disclosed herein (e.g., an AAV particle comprising an AAV capsid variant) may be administered to a subject by a delivery route, e.g., a localized delivery route or a systemic delivery route.

[0285] In some embodiments, an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) may be administered via such a route that it is able to cross the blood-brain barrier, vascular barrier, or other epithelial barrier. In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) may be administered in any suitable form, either as a liquid solution or suspension, as a solid form suitable for liquid solution or suspension in a liquid solution. In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant) may be formulated with any appropriate and pharmaceutically acceptable excipient.

[0286] In some embodiments, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) is administered intramuscularly, intravenously, intracerebrally, intrathecally, intratumorally, intracerebroventricularly, via intraparenchymal administration, or via intra-cisterna magna injection (ICM). In some embodiments, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) is administered intravenously. In some embodiments, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) is administered via intra-cisterna magna injection (ICM). In some embodiments, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) is administered intratumorally. In some embodiments, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) is administered intraarterially.

[0287] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) may be delivered to a subject via a single route administration. In some embodiments, an AAV particle of the present disclosure may be delivered to a subject via a multi-site route of administration. In some embodiments, a subject may be administered at 2, 3, 4, 5, or more than 5 sites.

[0288] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered via a bolus infusion. In some embodiments, an AAV particle of the present disclosure is administered via sustained delivery over a period of minutes, hours, or days. In some embodiments, the infusion rate may be changed depending on the subject, distribution, formulation, and/or another delivery parameter. In some embodiments, an AAV particle of the present disclosure is administered using a controlled release. In some embodiments, an AAV

particle of the present disclosure is administered using a sustained release, e.g., a release profile that conforms to a release rate over a specific period of time.

[0289] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant) may be delivered by more than one route of administration. As non-limiting examples of combination administrations, an AAV particle may be delivered by intrathecal and intracerebroventricular, or by intravenous and intraparenchymal administration.

Intravenous administration

[0290] In some embodiments, an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) may be administered to a subject by systemic administration. In some embodiments, the systemic administration is intravenous administration. In another embodiment, the systemic administration is intraarterial administration. In some embodiments, an AAV particle of the present disclosure may be administered to a subject by intravenous administration. In some embodiments, the intravenous administration may be achieved by subcutaneous delivery. In some embodiments, the AAV particle is administered to the subject via focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB) or MRI-guided FUS coupled with intravenous administration, e.g., as described in Terstappen et al. (Nat Rev Drug Discovery, doi.org/10.1038/s41573-021-00139-y (2021)), the contents of which are incorporated herein by reference in its entirety. In some embodiments, the AAV particle is administered to the subject intravenously. In some embodiments, the subject is a human.

Administration to the CNS

[0291] In some embodiments, an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) may be delivered by direct injection into the brain. As a non-limiting example, the brain delivery may be by intrahippocampal administration. In some embodiments, an AAV particle of the present disclosure may be administered to a subject by intraparenchymal administration. In some embodiments, the intraparenchymal administration is to tissue of the central nervous system. In some embodiments, an AAV particle of the present disclosure may be administered to a subject by intracranial delivery (See, e.g., US Pat. No. 8119611; the content of which is incorporated herein by reference in its entirety). In some embodiments, an AAV particle described herein may be delivered by injection into the CSF pathway. Non-limiting examples of delivery to the CSF pathway include intrathecal and intracerebroventricular administration. In some embodiments, an AAV particle described herein may be administered via intracisternal magna (ICM) injection.

[0292] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) may be delivered to the brain by systemic delivery. As a non-limiting example, the systemic delivery may be by intravascular administration. As a non-limiting example, the systemic or intravascular administration may be intravenous.

[0293] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant) of the present disclosure may be delivered by an intraocular delivery route. A non-limiting example of an intraocular administration includes an intravitreal injection.

Intramuscular administration

[0294] In some embodiments, an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) may be delivered by intramuscular administration. Without wishing to be bound by theory, it is believed in some embodiments, that the multi-nucleated nature of muscle cells provides an advantage to gene transduction subsequent to AAV delivery. In some embodiments, cells of the muscle are capable of expressing recombinant proteins with the appropriate post-translational modifications. Without wishing to be bound by theory, it is believed in some embodiments, the enrichment of muscle tissue with vascular structures allows for transfer to the blood stream and whole-body delivery. Examples of intramuscular administration include systemic (e.g., intravenous), subcutaneous or directly into the muscle. In some embodiments, more than one injection is administered. In some embodiments, an AAV particle of the present disclosure may be delivered by an intramuscular delivery route. (See, e.g., U. S. Pat. No. 6506379; the content of which is incorporated herein by reference in its entirety). Non-limiting examples of intramuscular administration include an intravenous injection or a subcutaneous injection.

[0295] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to a subject and transduces the muscle of a subject. As a non-limiting example, an AAV particle is administered by intramuscular administration. In some embodiments, an AAV particle of the present disclosure may be administered to a subject by subcutaneous administration. In some embodiments, the intramuscular administration is via systemic delivery. In some embodiments, the intramuscular administration is via intravenous delivery. In some embodiments, the intramuscular administration is via direct injection to the muscle.

[0296] In some embodiments, the muscle is transduced by administration, e.g., intramuscular administration. In some embodiments, an intramuscular delivery comprises administration at one site. In some embodiments, an intramuscular delivery comprises administration at more than one site. In some embodiments, an intramuscular delivery comprises administration at two, three, four, or more sites. In some embodiments, intramuscular delivery is combined with at least one other method of administration.

[0297] In some embodiments, an AAV particle pf the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) may be administered to a subject by peripheral injections. Non-limiting examples of peripheral injections include intraperitoneal, intramuscular, intravenous, conjunctival, or joint injection. It was disclosed in the art that the peripheral administration of AAV vectors can be transported to the central nervous system, for example, to the motor neurons (e.g., U.

S. Patent Publication Nos. US20100240739 and US20100130594; the content of each of which is incorporated herein by reference in their entirety).

[0298] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) may be administered to a subject by intraparenchymal administration. In some embodiments, the intraparenchymal administration is to muscle tissue. In some embodiments, an AAV particle of the present disclosure is delivered as described in Bright et al 2015 (Neurobiol Aging. 36(2):693-709), the contents of which are herein incorporated by reference in their entirety. In some embodiments, an AAV particle of the present disclosure is administered to the gastrocnemius muscle of a subject. In some embodiments, an AAV particle of the present disclosure is administered to the bicep femorii of the subject. In some embodiments, an AAV particles of the present disclosure is administered to the tibialis anterior muscles. In some embodiments, an AAV particle of the present disclosure is administered to the soleus muscle.

Depot administration

[0299] In some embodiments, a pharmaceutical composition and/or an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) are formulated in depots for extended release. Generally, specific organs or tissues are targeted for administration.

[0300] In some embodiments, a pharmaceutical composition and/or an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) are spatially retained within or proximal to target tissues. Provided are methods of providing a pharmaceutical composition, an AAV particle, to target tissues of mammalian subjects by contacting target tissues (which comprise one or more target cells) with the pharmaceutical composition and/or the AAV particle, under conditions such that they are substantially retained in target tissues, e.g., such that at least 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the composition is retained in the target tissues. In some embodiments, retention is determined by measuring the amount of pharmaceutical composition and/or AAV particle, that enter a target cell or a plurality of target cells. For example, at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, or greater than 99.99% of a pharmaceutical composition and/or an AAV particle, administered to a subject are present intracellularly at a period of time following administration. For example, intramuscular injection to a subject may be performed using aqueous compositions comprising a pharmaceutical composition and/or an AAV particle of the present disclosure and a transfection reagent, and retention is determined by measuring the amount of the pharmaceutical composition and/or the AAV particle, present in the muscle cell or plurality of muscle cells.

[0301] In some embodiments, disclosed herein are methods of providing a pharmaceutical composition and/or an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) to a tissue of a subject, by contacting the tissue (comprising a cell, e.g., a

plurality of cells) with the pharmaceutical composition and/or the AAV particle under conditions such that they are substantially retained in the tissue. In some embodiments, a pharmaceutical composition and/or AAV particle described herein comprise a sufficient amount of an active ingredient such that the effect of interest is produced in at least one cell. In some embodiments, a pharmaceutical composition and/or an AAV particle generally comprise one or more cell penetration agents. In some embodiments, the disclosure provides a naked formulations (such as without cell penetration agents or other agents), with or without pharmaceutically acceptable carriers.

Methods of Treatment

[0302] Provided in the present disclosure are methods for introducing (e.g., delivering) an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) into cells. In some embodiments, the method comprises introducing into said cells an AAV particle or vector described herein in an amount sufficient to modulate, e.g., increase, the production of a target gene, mRNA, and/or protein. In some embodiments, the method comprises introducing into said cells an AAV particle or vector described herein in an amount sufficient to modulate, e.g., decrease, expression of a target gene, mRNA, and/or protein. In some aspects, the cells may be neurons such as but not limited to, motor, hippocampal, entorhinal, thalamic, cortical, sensory, sympathetic, or parasympathetic neurons, and glial cells such as astrocytes, microglia, and/or oligodendrocytes. In other aspects, the cells may be a muscle cell, e.g., a cell of a diaphragm, a quadriceps, or a heart (e.g., a heart atrium or a heart ventricle). In other embodiments, the cells may be a muscle cell (e.g., a cell of a diaphragm, a quadriceps, or a heart (e.g., a heart atrium or a heart ventricle)) or a liver cell. In some embodiments, the cell may be a heart cell (e.g., a cell of a heart atrium or a cell of a heart ventricle).

[0303] Disclosed in the present disclosure are methods for treating a neurological disease/disorder or a neurodegenerative disorder, a muscular or neuromuscular disorder, or a neurooncological disorder associated with aberrant, e.g., insufficient or increased, function/presence of a protein, e.g., a target protein in a subject in need of treatment.

[0304] In some embodiments, the method comprises administering to the subject a therapeutically effective amount of a composition comprising AAV particles of the present disclosure. As a non-limiting example, the AAV particles can increase target gene expression, increase target protein production, and thus reduce one or more symptoms of neurological disease in the subject such that the subject is therapeutically treated.

[0305] In other embodiments, the method comprises administering to the subject a therapeutically effective amount of a composition comprising AAV particles (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) comprising a viral genome with a nucleic acid sequence encoding one or more siRNA molecules. As a non-limiting example, the siRNA molecules

can silence target gene expression, inhibit target protein production, and reduce one or more symptoms of neurological disease in the subject such that the subject is therapeutically treated.

[0306] In some embodiments, the composition comprising the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant described herein) is administered to the central nervous system of the subject via systemic administration. In some embodiments, the systemic administration is intravenous (IV) injection. In some embodiments, the AAV particle described herein or a pharmaceutical composition comprising an AAV particle described herein is administered by focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB) or MRI-guided FUS coupled with intravenous administration.

[0307] In some embodiments, the composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject via intraventricular administration. In some embodiments, the composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered via intra-cisterna magna injection (ICM).

[0308] In some embodiments, the composition comprising an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject via intraventricular injection and intravenous injection.

[0309] In some embodiments, the composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject via ICM injection and intravenous injection at a specific dose per subject. As a non-limiting example, the AAV particles are administered via ICM injection at a dose of $1x10^{4}$ VG per subject. As a non-limiting example, the AAV particles are administered via IV injection at a dose of $2x10^{13}$ VG per subject.

[0310] In some embodiments, the composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject. In other embodiments, the composition comprising the AAV particles of the present disclosure is administered to a CNS tissue of a subject (e.g., putamen, hippocampus, thalamus, or cortex of the subject).

[0311] In some embodiments, the composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject via intraparenchymal injection. Non-limiting examples of intraparenchymal injections include intraputamenal, intracortical, intrathalamic, intrastriatal, intrahippocampal or into the entorhinal cortex.

[0312] In some embodiments, the composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject via intraparenchymal injection and intravenous injection.

[0313] In some embodiments, the composition comprising the AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) is administered to the central nervous system of the subject via intraventricular injection, intraparenchymal injection and intravenous injection.

[0314] In some embodiments, the composition comprising an AAV particle (e.g., an AAV particle comprising an AAV capsid variant) of a plurality of particles of the present disclosure is administered to a muscle of the subject via intravenous injection. In some embodiments, the composition comprising an AAV particle of a plurality of particles of the present disclosure is administered to a muscle of the subject via intramuscular injection.

[0315] In some embodiments, an AAV particle of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) may be delivered into specific types of cells, including, but not limited to, thalamic, hippocampal, entorhinal, cortical, motor, sensory, excitatory, inhibitory, sympathetic, or parasympathetic neurons; glial cells including oligodendrocytes, astrocytes and microglia; and/or other cells surrounding neurons such as T cells. In some embodiments, an AAV particle of the present disclosure may be delivered into a muscle cell, e.g., a cell of the quadriceps, diaphragm, liver, and/or heart (e.g., heart atrium or heart ventricle).

[0316] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be delivered to a cell or region of the midbrain. In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be delivered to a cell or region of the brains stem.

[0317] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be delivered to neurons in the putamen, hippocampus, thalamus and/or cortex.

[0318] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for a genetic disorder, e.g., an autosomal dominant genetic disorder, an autosomal recessive disorder, X-linked dominant genetic disorder, an X-linked recessive genetic disorder, or a Y-linked genetic disorder. In some embodiments, the genetic disorder is a monogenetic disorder or a polygenic disorder. In some embodiments, treatment of a genetic disorder, e.g., a monogenic disorder, comprises the use of an AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant described herein) for a gene replacement therapy.

[0319] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for a neurological disease.

[0320] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for tauopathies.

[0321] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for Alzheimer's Disease.

[0322] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for Amyotrophic Lateral Sclerosis.

[0323] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for Huntington's Disease.

[0324] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for Parkinson's Disease.

[0325] n some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for Gaucher disease (GD) (e.g., Type 1 GD, Type 2 GD, or Type 3 GD). In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for Parkinson's disease. In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for dementia with Lewy Bodies (DLB).

[0326] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for spinal muscular atrophy.

[0327] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for a leukodystrophy, e.g., Alexander disease, autosomal dominant leukodystrophy with autonomic diseases (ADLD), Canavan disease, cerebrotendinous xanthomatosis (CTX), metachromatic leukodystrophy (MLD), Pelizaeus-Merzbacher disease, or Refsum disease.

[0328] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for Friedreich's Ataxia.

[0329] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for chronic or neuropathic pain.

[0330] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for a muscular disorder or a neuromuscular disorder.

[0331] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for a cardiac disease or heart disease and/or method of improving (e.g., enhancing) cardiac function in a subject. In some embodiments, the cardiac disease is a cardiomyopathy (e.g., arrhythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy, or hypertrophic cardiomyopathy), congestive heart failure, tachycardia (e.g., catecholaminergic polymorphic ventricular tachycardia), ischemic heart disease, and/or myocardial infarction.

[0332] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant), e.g., a plurality of particles, of the present disclosure may be used as a therapy for a disease associated with expression of HER2, e.g., a disease associated with overexpression of HER2. In some embodiments, the AAV particle of the disclosure (e.g., an AAV particle comprising an AAV capsid variant) is useful for the treatment, prophylaxis, palliation or amelioration of a HER2-positive cancer. In some embodiments, the HER2-positive cancer is a HER2-positive solid tumor. Additionally, or alternatively, the HER2-positive cancer may be a locally advanced or metastatic HER2-positive cancer. In some instances, the HER2-positive cancer is a HER2-positive breast cancer or a HER2-positive gastric cancer. In some embodiments, the HER2-positive cancer is selected from the group consisting of a HER2- positive gastroesophageal junction cancer, a HER2-positive colorectal cancer, a HER2-positive lung cancer (e.g., a HER2-positive non-small cell lung carcinoma), a HER2-positive pancreatic cancer, a HER2-positive colorectal cancer, a HER2-positive bladder cancer, a HER2-positive salivary duct cancer, a HER2-positive ovarian cancer (e.g., a HER2-positive epithelial ovarian cancer), or a HER2-positive endometrial cancer. In some instances, the HER2-positive cancer is prostate cancer. In some embodiments, the HER2-positive cancer has metastasized to the central nervous system (CNS). In some instances, the metastasized HER2-cancer has formed CNS neoplasms.

[0333] In some embodiments, an AAV particle (e.g., an AAV particle comprising an AAV capsid variant) e.g., a plurality of particles, of the present disclosure may be used as a therapy for a neuro-oncological disorder. In some embodiments, the neuro-oncological disorder is a cancer of primary CNS origin (e.g., a cancer of a CNS cell and/or CNS tissue). In some embodiments, the neuro-oncological disorder is metastatic cancer in a CNS cell, CNS region, and/or a CNS tissue. Examples of primary CNS cancers could be gliomas (which may include glioblastoma (also known as glioblastoma multiforme), astrocytomas, oligodendrogliomas, and ependymomas, and mixed gliomas), meningiomas, medulloblastomas, neuromas, and primary CNS lymphoma (in the brain, spinal cord, or meninges), among others. Examples of metastatic cancers include those originating in

another tissue or organ, e.g., breast, lung, lymphoma, leukemia, melanoma (skin cancer), colon, kidney, prostate, or other types that metastasize to brain.

[0334] In some embodiments, administration of the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) to a subject may increase target gene, mRNA, and/or protein levels in a subject, relative to a control, e.g., the gene, mRNA, and/or mRNA levels in the subject prior to receiving AAV particle. The target gene, mRNA, and/or protein levels may be increased by about 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100% in a subject such as, but not limited to, the CNS, a region of the CNS, or a specific cell of the CNS, or a muscle, a region of a muscle, or a cell of a muscle, of a subject. In some embodiments, cell of the CNS comprises an astrocyte, microglia, cortical neuron, hippocampal neuron, DRG and/or sympathetic neuron, sensory neuron, oligodendrocyte, motor neuron, or combination thereof. As a non-limiting example, the AAV particles may increase the gene, mRNA, and/or protein levels of a target protein by fold increases over baseline. In some embodiments, AAV particles lead to 5-6 times higher levels of a target gene, mRNA, or protein.

[0335] In some embodiments, administration of the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant), e.g., an AAV particle comprising a nucleic acid encoding a siRNA molecule, to a subject may decrease target gene, mRNA, and/or protein levels in a subject, relative to a control, e.g., the gene, mRNA, and/or mRNA levels in the subject prior to receiving AAV particle. The target gene, mRNA, and/or protein levels may be decreased by about 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%, or at least 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-95%, 20-100%, 30-40%, 30-50%, 30-60%, 30-70%, 30-80%, 30-90%, 30-95%, 30-100%, 40-50%, 40-60%, 40-70%, 40-80%, 40-90%, 40-95%, 40-100%, 50-60%, 50-70%, 50-80%, 50-90%, 50-95%, 50-100%, 60-70%, 60-80%, 60-90%, 60-95%, 60-100%, 70-80%, 70-90%, 70-95%, 70-100%, 80-90%, 80-95%, 80-100%, 90-95%, 90-100% or 95-100% in a subject such as, but not limited to, the CNS, a region of the CNS, or a specific cell of the CNS, or a muscle, a region of a muscle, or a cell of a muscle, of a subject. In some embodiments, cell of the CNS comprises an astrocyte, microglia, cortical neuron, hippocampal neuron, DRG and/or sympathetic neuron, sensory neuron, oligodendrocyte, motor neuron, or combination thereof. As a non-limiting example, the AAV particles may decrease the gene, mRNA, and/or protein levels of a target protein by fold decreases over baseline.

[0336] In some embodiments, the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) may be used to increase target protein and reduce symptoms of neurological disease in a subject. In some embodiments, the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) may be used to decrease target protein and reduce symptoms of neurological disease in a subject.

[0337] In some embodiments, the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) may be used to reduce the decline of functional capacity and activities of daily living as measured by a standard evaluation system such as, but not limited to, the total functional capacity (TFC) scale.

[0338] In some embodiments, the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid polypeptide, e.g., an AAV capsid variant) may be used to improve performance on any assessment used to measure symptoms of neurological disease. Such assessments include, but are not limited to ADAS-cog (Alzheimer Disease Assessment Scale – cognitive), MMSE (Mini-Mental State Examination), GDS (Geriatric Depression Scale), FAQ (Functional Activities Questionnaire), ADL (Activities of Daily Living), GPCOG (General Practitioner Assessment of Cognition), Mini-Cog, AMTS (Abbreviated Mental Test Score), Clock-drawing test, 6-CIT (6-item Cognitive Impairment Test), TYM (Test Your Memory), MoCa (Montreal Cognitive Assessment), ACE-R (Addenbrookes Cognitive Assessment), MIS (Memory Impairment Screen), BADLS (Bristol Activities of Daily Living Scale), Barthel Index, Functional Independence Measure, Instrumental Activities of Daily Living, IQCODE (Informant Questionnaire on Cognitive Decline in the Elderly), Neuropsychiatric Inventory, The Cohen-Mansfield Agitation Inventory, BEHAVE-AD, EuroQol, Short Form-36 and/or MBR Caregiver Strain Instrument, or any of the other tests as described in Sheehan B (Ther Adv Neurol Disord. 5(6):349-358 (2012)), the contents of which are herein incorporated by reference in their entirety.

[0339] In some embodiments, the present composition is administered as a solo therapeutic or as combination therapeutic for the treatment of a neurological disease/disorder or a neurodegenerative disorder, a muscular disorder or neuromuscular disorder, and/or a neuro-oncological disorder.

[0340] The AAV particles (e.g., an AAV particle comprising an AAV capsid variant) encoding the target protein may be used in combination with one or more other therapeutic agents. In some embodiments, compositions can be administered concurrently with, prior to, or subsequent to, additional therapeutic or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent.

[0341] Therapeutic agents that may be used in combination with the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) can be small molecule compounds which are antioxidants, anti-inflammatory agents, anti-apoptosis agents, calcium

regulators, anti-glutamatergic agents, structural protein inhibitors, compounds involved in muscle function, and compounds involved in metal ion regulation. As a non-limiting example, the combination therapy may be in combination with one or more neuroprotective agents such as small molecule compounds, growth factors and hormones which have been tested for their neuroprotective effect on motor neuron degeneration.

[0342] Compounds tested for treating neurological disease which may be used in combination with the AAV particles described herein include, but are not limited to, cholinesterase inhibitors (donepezil, rivastigmine, galantamine), NMDA receptor antagonists such as memantine, anti-psychotics, anti-depressants, anti-convulsants (e.g., sodium valproate and levetiracetam for myoclonus), secretase inhibitors, amyloid aggregation inhibitors, copper or zinc modulators, BACE inhibitors, inhibitors of tau aggregation, such as Methylene blue, phenothiazines, anthraquinones, n-phenylamines or rhodamines, microtubule stabilizers such as NAP, taxol or paclitaxel, kinase or phosphatase inhibitors such as those targeting GSK3β (lithium) or PP2A, immunization with Aβ peptides or tau phospho-epitopes, anti-tau or anti-amyloid antibodies, dopamine-depleting agents (e.g., tetrabenazine for chorea), benzodiazepines (e.g., clonazepam for myoclonus, chorea, dystonia, rigidity, and/or spasticity), amino acid precursors of dopamine (e.g., levodopa for rigidity), skeletal muscle relaxants (e.g., baclofen, tizanidine for rigidity and/or spasticity), inhibitors for acetylcholine release at the neuromuscular junction to cause muscle paralysis (e.g., botulinum toxin for bruxism and/or dystonia), atypical neuroleptics (e.g., olanzapine and quetiapine for psychosis and/or irritability, risperidone, sulpiride and haloperidol for psychosis, chorea and/or irritability, clozapine for treatment-resistant psychosis, aripiprazole for psychosis with prominent negative symptoms), selective serotonin reuptake inhibitors (SSRIs) (e.g., citalopram, fluoxetine, paroxetine, sertraline, mirtazapine, venlafaxine for depression, anxiety, obsessive compulsive behavior and/or irritability), hypnotics (e.g., xopiclone and/or zolpidem for altered sleep-wake cycle), anticonvulsants (e.g., sodium valproate and carbamazepine for mania or hypomania) and mood stabilizers (e.g., lithium for mania or hypomania).

[0343] Neurotrophic factors may be used in combination therapy with the AAV particles of the present disclosure (e.g., an AAV particle comprising an AAV capsid variant) for treating neurological disease. Generally, a neurotrophic factor is defined as a substance that promotes survival, growth, differentiation, proliferation and/or maturation of a neuron, or stimulates increased activity of a neuron. In some embodiments, the present methods further comprise delivery of one or more trophic factors into the subject in need of treatment. Trophic factors may include, but are not limited to, IGF-I, GDNF, BDNF, CTNF, VEGF, Colivelin, Xaliproden, Thyrotrophin-releasing hormone and ADNF, and variants thereof.

[0344] In one aspect, the AAV particle described herein (e.g., an AAV particle comprising an AAV capsid variant) may be co-administered with AAV particles expressing neurotrophic factors

such as AAV-IGF-I (See e.g., Vincent et al., *Neuromolecular medicine*, 2004, 6, 79-85; the contents of which are incorporated herein by reference in their entirety) and AAV-GDNF (See e.g., Wang et al., *J Neurosci.*, 2002, 22, 6920-6928; the contents of which are incorporated herein by reference in their entirety).

[0345] In some embodiments, administration of the AAV particles (e.g., an AAV particle comprising an AAV capsid variant) to a subject will modulate, e.g., increase or decrease, the expression of a target protein in a subject and the modulation, e.g., increase or decrease of the presence, level, activity, and/or expression of the target protein will reduce the effects and/or symptoms of a neurological disease/disorder or a neurodegenerative disorder, a muscular disorder or neuromuscular disorder, and/or a neuro-oncological disorder in a subject.

DEFINITIONS

[0346] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

[0347] Articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The disclosure includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The disclosure includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process.

[0348] It is also noted that the term "comprising" is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term "comprising" is used herein, the term "consisting of" and "consisting essentially thereof" is thus also encompassed and disclosed.

[0349] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[0350] *Adeno-associated virus:* As used herein, the term "adeno-associated virus" or "AAV" refers to members of the dependovirus genus or a variant, e.g., a functional variant, thereof. In some embodiments, the AAV is wildtype, or naturally occurring. In some embodiments, the AAV is recombinant.

[0351] *AAV Particle*: As used herein, an "AAV particle" refers to a particle or a virion comprising an AAV capsid, e.g., an AAV capsid variant, and a polynucleotide, e.g., a viral genome or a vector genome. In some embodiments, the viral genome of the AAV particle comprises at least one payload

region and at least one ITR. In some embodiments, an AAV particle of the disclosure is an AAV particle comprising an AAV variant. In some embodiments, the AAV particle is capable of delivering a nucleic acid, e.g., a payload region, encoding a payload to cells, typically, mammalian, e.g., human, cells. In some embodiments, an AAV particle of the present disclosure may be produced recombinantly. In some embodiments, an AAV particle may be derived from any serotype, described herein or known in the art, including combinations of serotypes (e.g., "pseudotyped" AAV) or from various genomes (e.g., single stranded or self-complementary). In some embodiments, the AAV particle may be replication defective and/or targeted. It is to be understood that reference to the AAV particle of the disclosure also includes pharmaceutical compositions thereof, even if not explicitly recited.

[0352] *Administering:* As used herein, the term "administering" refers to providing a pharmaceutical agent or composition to a subject.

[0353] *Amelioration*: As used herein, the term "amelioration" or "ameliorating" refers to a lessening of severity of at least one indicator of a condition or disease. For example, in the context of neurodegeneration disorder, amelioration includes the reduction of neuron loss.

[0354] *Amplicon*: As used herein, "amplicon" may refer to any piece of RNA or DNA formed as the product of amplification events, e.g. PCR. In some embodiments, full-length capsid amplicons may be used as templates for next generation sequencing (NGS) library generation. Full-length capsid amplicons may be used for cloning into a DNA library for any number of additional rounds of AAV selection as described herein.

[0355] *Animal:* As used herein, the term "animal" refers to any member of the animal kingdom. In some embodiments, "animal" refers to humans at any stage of development. In some embodiments, "animal" refers to non-human animals at any stage of development. In certain embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, and worms. In some embodiments, the animal is a transgenic animal, genetically engineered animal, or a clone.

[0356] *Antisense strand:* As used herein, the term "the antisense strand" or "the first strand" or "the guide strand" of a siRNA molecule refers to a strand that is substantially complementary to a section of about 10-50 nucleotides, e.g., about 15-30, 16-25, 18-23 or 19-22 nucleotides of the mRNA of a gene targeted for silencing. The antisense strand or first strand has sequence sufficiently complementary to the desired target mRNA sequence to direct target-specific silencing, e.g., complementarity sufficient to trigger the destruction of the desired target mRNA by the RNAi machinery or process.

[0357] *Approximately:* As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain

embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0358] *Biopanning:* As used herein, the term "biopanning" refers to an AAV capsid library selection process comprising administration of an AAV particle with enhanced tissue- and/or cell type-specific transduction to a cell and/or subject; extraction of nucleotides encoded by said AAV particle from said transduced tissue- and/or cell type-specific; and, use of the extracted nucleotides for cloning into a nucleotide library for the generation of AAV particles for subsequent rounds of the same.

[0359] *Capsid*: As used herein, the term "capsid" refers to the exterior, e.g., a protein shell, of a virus particle, e.g., an AAV particle, that is substantially (e.g., >50%, >60%, >70%, >80%, >90%, >95%, >99%, or 100%) protein. In some embodiments, the capsid is an AAV capsid comprising an AAV capsid protein described herein, e.g., a VP1, VP2, and/or VP3 polypeptide. The AAV capsid protein can be a wild-type AAV capsid protein or a variant, e.g., a structural and/or functional variant from a wild-type or a reference capsid protein, referred to herein as an "AAV capsid variant." In some embodiments, the AAV capsid variant described herein has the ability to enclose, e.g., encapsulate, a viral genome and/or is capable of entry into a cell, e.g., a mammalian cell. In some embodiments, the AAV capsid variant described herein may have modified tropism compared to that of a wild-type AAV capsid, e.g., the corresponding wild-type capsid.

[0360] *Complementary and substantially complementary:* As used herein, the term "complementary" refers to the ability of polynucleotides to form base pairs with one another. Base pairs are typically formed by hydrogen bonds between nucleotide units in antiparallel polynucleotide strands. Complementary polynucleotide strands can form base pairs in the Watson-Crick manner (e.g., A to T, A to U, C to G), or in any other manner that allows for the formation of duplexes. As persons skilled in the art are aware, when using RNA as opposed to DNA, uracil rather than thymine is the base that is considered to be complementary to adenine. However, when a U is denoted in the context of the present disclosure, the ability to substitute a T is implied, unless otherwise stated. Perfect complementarity or 100% complementarity refers to the situation in which each nucleotide unit of one polynucleotide strand can form a hydrogen bond with a nucleotide unit of a second polynucleotide strand. Less than perfect complementarity refers to the situation in which some, but not all, nucleotide units of two strands can form hydrogen bond with each other. For example, for two 20-mers, if only two base pairs on each strand can form a hydrogen bond with each other, the polynucleotide strands exhibit 10% complementarity. In the same example, if 18 base pairs on each strand can form hydrogen bonds with each other, the polynucleotide strands exhibit 90% complementarity. The term

"complementary" as used herein can encompass fully complementary, partially complementary, or substantially complementary. As used herein, the term "substantially complementary" means that the siRNA has a sequence (e.g., in the antisense strand) which is sufficient to bind the desired target mRNA, and to trigger the RNA silencing of the target mRNA. "Fully complementary", "perfect complementarity", or "100% complementarity" refers to the situation in which each nucleotide unit of one polynucleotide or oligonucleotide strand can base-pair with a nucleotide unit of a second polynucleotide or oligonucleotide strand.

[0361] *Control Elements:* As used herein, "control elements", "regulatory control elements" or "regulatory sequences" refers to promoter regions, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, and the like, which provide for the replication, transcription and translation of a coding sequence in a recipient cell. Not all of these control elements need always be present as long as the selected coding sequence is capable of being replicated, transcribed and/or translated in an appropriate host cell.

[0362] *Delivery:* As used herein, "delivery" refers to the act or manner of delivering an AAV particle, a compound, substance, entity, moiety, cargo or payload.

[0363] *Element:* As used herein, the term "element" refers to a distinct portion of an entity. In some embodiments, an element may be a polynucleotide sequence with a specific purpose, incorporated into a longer polynucleotide sequence.

[0364] *Encapsulate:* As used herein, the term "encapsulate" means to enclose, surround or encase. As an example, a capsid protein, e.g., an AAV capsid variant, often encapsulates a viral genome. In some embodiments, encapsulate within a capsid, e.g., an AAV capsid variant, encompasses 100% coverage by a capsid, as well as less than 100% coverage, e.g., 95%, 90%, 85%, 80%, 70%, 60% or less. For example, gaps or discontinuities may be present in the capsid so long as the viral genome is retained in the capsid, e.g., prior to entry into a cell.

[0365] *Effective Amount:* As used herein, the term "effective amount" of an agent is that amount sufficient to effect beneficial or desired results, for example, clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied. For example, in the context of administering an agent that treats cancer, an effective amount of an agent is, for example, an amount sufficient to achieve treatment, as defined herein, of cancer, as compared to the response obtained without administration of the agent.

[0366] *Expression*: As used herein, "expression" of a nucleic acid sequence refers to one or more of the following events: (1) production of an RNA template from a DNA sequence (*e.g.*, by transcription); (2) processing of an RNA transcript (*e.g.*, by splicing, editing, 5′ cap formation, and/or 3′ end processing); (3) translation of an RNA into a polypeptide or protein; and (4) post-translational modification of a polypeptide or protein.

[0367] *Formulation*: As used herein, a "formulation" includes at least one AAV particle (active ingredient) and an excipient, and/or an inactive ingredient.

[0368] *Fragment:* A "fragment," as used herein, refers to a portion. For example, an antibody fragment may comprise a CDR, or a heavy chain variable region, or a scFv, etc.

[0369] *Homology*: As used herein, the term "homology" refers to the overall relatedness between polymeric molecules, *e.g.* between polynucleotide molecules (*e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be "homologous" to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical or similar. The term "homologous" necessarily refers to a comparison between at least two sequences (polynucleotide or polypeptide sequences). In accordance with the disclosure, two polynucleotide sequences are considered to be homologous if the polypeptides they encode are at least about 50%, 60%, 70%, 80%, 90%, 95%, or even 99% for at least one stretch of at least about 20 amino acids. In some embodiments, homologous polynucleotide sequences are characterized by the ability to encode a stretch of at least 4–5 uniquely specified amino acids. For polynucleotide sequences less than 60 nucleotides in length, homology is determined by the ability to encode a stretch of at least 4–5 uniquely specified amino acids. In accordance with the disclosure, two protein sequences are considered to be homologous if the proteins are at least about 50%, 60%, 70%, 80%, or 90% identical for at least one stretch of at least about 20 amino acids.

[0370] *Identity*: As used herein, the term "identity" refers to the overall relatedness between polymeric molecules, *e.g.*, between polynucleotide molecules (*e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of the percent identity of two polynucleotide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using methods such as those described in Computational Molecular Biology, Lesk, A. M.,

ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; the contents of each of which are incorporated herein by reference in their entirety. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4:11-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix. Methods commonly employed to determine percent identity between sequences include, but are not limited to those disclosed in Carillo, H., and Lipman, D., SIAM J Applied Math., 48:1073 (1988); incorporated herein by reference. Techniques for determining identity are codified in publicly available computer programs. Exemplary computer software to determine homology between two sequences include, but are not limited to, GCG program package, Devereux, J., *et al.*, *Nucleic Acids Research*, 12(1), 387 (1984)), BLASTP, BLASTN, and FASTA Altschul, S. F. *et al.*, *J. Molec. Biol.*, 215, 403 (1990)).

[0371] *Inhibit expression of a gene:* As used herein, the phrase "inhibit expression of a gene" means to cause a reduction in the amount of an expression product of the gene. The expression product can be an RNA transcribed from the gene (*e.g.*, an mRNA) or a polypeptide translated from an mRNA transcribed from the gene. Typically, a reduction in the level of an mRNA results in a reduction in the level of a polypeptide translated therefrom. The level of expression may be determined using standard techniques for measuring mRNA or protein.

[0372] *Inverted terminal repeat:* As used herein, the term "inverted terminal repeat" or "ITR" refers to a cis-regulatory element for the packaging of polynucleotide sequences into viral capsids.

[0373] *Isolated*: As used herein, the term "isolated" refers to a substance or entity that is altered or removed from the natural state, e.g., altered or removed from at least some of component with which it is associated in the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of the environment in which it is found in nature. In some embodiments, an isolated nucleic acid is recombinant, e.g., incorporated into a vector.

[0374] *Library:* As used herein, the term "library" refers to a diverse collection of linear polypeptides, polynucleotides, viral particles, or viral vectors. As examples, a library may be a DNA library or an AAV capsid library.

[0375] *Molecular scaffold:* As used herein a "molecular scaffold" is a framework or starting molecule that forms the sequence or structural basis against which to design or make a subsequent molecule.

[0376] *Neurological disease:* As used herein, a "neurological disease" is any disease associated with the central or peripheral nervous system and components thereof (e.g., neurons).

[0377] *Orthogonal evolution:* As used herein, the term "orthogonal evolution" refers to a method wherein AAV particles are administered for a first round of AAV selection as described herein across a set of any number of cell- and/or subject-types that may be from different species and/or strains, and wherein any number of additional, i.e., subsequent, AAV selection rounds are performed either across a set of any number of cell- and/or subject-types that may be from different species and/or strains, or across a set of any number of cell- and/or subject-types that may be from the same species and/or strain.

[0378] *Open reading frame:* As used herein, "open reading frame" or "ORF" refers to a sequence which does not contain a stop codon in a given reading frame.

[0379] *Particle*: As used herein, a "particle" is a virus comprised of at least two components, a protein capsid and a polynucleotide sequence enclosed within the capsid.

[0380] *Payload region:* As used herein, a "payload region" is any nucleic acid sequence (e.g., within the viral genome) which encodes one or more "payloads" of the disclosure. As non-limiting examples, a payload region may be a nucleic acid sequence within the viral genome of an AAV particle, which encodes a payload, wherein the payload is an RNAi agent or a polypeptide. Payloads of the present disclosure may be, but are not limited to, peptides, polypeptides, proteins, antibodies, RNAi agents, etc.

[0381] *Polypeptide:* As used herein, "polypeptide" means a polymer of amino acid residues (natural or unnatural) linked together most often by peptide bonds. The term, as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. In some instances, the polypeptide encoded is smaller than about 50 amino acids and the polypeptide is then termed a peptide. If the polypeptide is a peptide, it will be at least about 2, 3, 4, or at least 5 amino acid residues long. Thus, polypeptides include gene products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing. A polypeptide may be a single molecule or may be a multi-molecular complex such as a dimer, trimer or tetramer. They may also comprise single chain or multichain polypeptides and may be associated or linked. The term polypeptide may also apply to amino acid polymers in which one or

more amino acid residues are an artificial chemical analogue of a corresponding naturally occurring amino acid.

[0382] *Polypeptide variant:* The term "polypeptide variant" refers to molecules which differ in their amino acid sequence from a native or reference sequence. The amino acid sequence variants may possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence, as compared to a native or reference sequence. In some embodiments, a variant comprises a sequence having at least about 50%, at least about 80%, or at least about 90%, identical (homologous) to a native or a reference sequence.

[0383] *Peptide:* As used herein, "peptide" is less than or equal to 50 amino acids long, e.g., about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

[0384] *Pharmaceutically acceptable*: The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0385] *Preventing*: As used herein, the term "preventing" or "prevention" refers to partially or completely delaying onset of an infection, disease, disorder and/or condition; partially or completely delaying onset of one or more symptoms, features, or clinical manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying onset of one or more symptoms, features, or manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying progression from an infection, a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the infection, the disease, disorder, and/or condition.

[0386] *Prophylactic*: As used herein, "prophylactic" refers to a therapeutic or course of action used to prevent the spread of disease.

[0387] *Prophylaxis:* As used herein, a "prophylaxis" refers to a measure taken to maintain health and prevent the spread of disease.

[0388] *Region:* As used herein, the term "region" refers to a zone or general area. In some embodiments, when referring to a protein or protein module, a region may comprise a linear sequence of amino acids along the protein or protein module or may comprise a three-dimensional area, an epitope and/or a cluster of epitopes. In some embodiments, regions comprise terminal regions. As used herein, the term "terminal region" refers to regions located at the ends or termini of a given agent. When referring to proteins, terminal regions may comprise N- and/or C-termini.

[0389] In some embodiments, when referring to a polynucleotide, a region may comprise a linear sequence of nucleic acids along the polynucleotide or may comprise a three-dimensional area, secondary structure, or tertiary structure. In some embodiments, regions comprise terminal regions.

As used herein, the term "terminal region" refers to regions located at the ends or termini of a given agent. When referring to polynucleotides, terminal regions may comprise 5' and/or 3' termini.

[0390] *RNA or RNA molecule*: As used herein, the term "RNA" or "RNA molecule" or "ribonucleic acid molecule" refers to a polymer of ribonucleotides; the term "DNA" or "DNA molecule" or "deoxyribonucleic acid molecule" refers to a polymer of deoxyribonucleotides. DNA and RNA can be synthesized naturally, e.g., by DNA replication and transcription of DNA, respectively; or be chemically synthesized. DNA and RNA can be single-stranded (i.e., ssRNA or ssDNA, respectively) or multi-stranded (e.g., double stranded, i.e., dsRNA and dsDNA, respectively). The term "mRNA" or "messenger RNA", as used herein, refers to a single stranded RNA that encodes the amino acid sequence of one or more polypeptide chains.

[0391] *RNA interfering or RNAi:* As used herein, the term "RNA interfering" or "RNAi" refers to a sequence specific regulatory mechanism mediated by RNA molecules which results in the inhibition or interfering or "silencing" of the expression of a corresponding protein-coding gene. RNAi has been observed in many types of organisms, including plants, animals and fungi. RNAi occurs in cells naturally to remove foreign RNAs (e.g., viral RNAs). Natural RNAi proceeds via fragments cleaved from free dsRNA which direct the degradative mechanism to other similar RNA sequences. RNAi is controlled by the RNA-induced silencing complex (RISC) and is initiated by short/small dsRNA molecules in cell cytoplasm, where they interact with the catalytic RISC component argonaute. The dsRNA molecules can be introduced into cells exogenously. Exogenous dsRNA initiates RNAi by activating the ribonuclease protein Dicer, which binds and cleaves dsRNAs to produce double-stranded fragments of 21-25 base pairs with a few unpaired overhang bases on each end. These short double stranded fragments are called small interfering RNAs (siRNAs).

[0392] *RNAi agent:* As used herein, the term "RNAi agent" refers to an RNA molecule, or its derivative, that can induce inhibition, interfering, or "silencing" of the expression of a target gene and/or its protein product. An RNAi agent may knock-out (virtually eliminate or eliminate) expression, or knock-down (lessen or decrease) expression. The RNAi agent may be, but is not limited to, dsRNA, siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA.

[0393] *miR binding site:* As used herein, a "miR binding site" comprises a nucleic acid sequence (whether RNA or DNA, e.g., differ by "U" of RNA or "T" in DNA) that is capable of binding, or binds, in whole or in part to a microRNA (miR) through complete or partial hybridization . Typically, such binding occurs between the miR and the miR binding site in the reverse complement orientation. In some embodiments, the miR binding site is transcribed from the AAV vector genome encoding the miR binding site.

[0394] In some embodiments, a miR binding site may be encoded or transcribed in series. Such a "miR binding site series" or "miR BSs" may include two or more miR binding sites having the same or different nucleic acid sequence.

[0395] *Spacer*: As used here, a "spacer" is generally any selected nucleic acid sequence of, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length, which is located between two or more consecutive miR binding site sequences. Spacers may also be more than 10 nucleotides in length, e.g., 20, 30, 40, or 50 or more than 50 nucleotides.

[0396] *Sample:* As used herein, the term "sample" or "biological sample" refers to a subset of its tissues, cells, nucleic acids, or component parts (e.g. body fluids, including but not limited to blood, serum, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen).

[0397] *Self-complementary viral particle*: As used herein, a "self-complementary viral particle" is a particle comprised of at least two components, a protein capsid and a self-complementary viral genome enclosed within the capsid.

[0398] *Sense Strand:* As used herein, the term "the sense strand" or "the second strand" or "the passenger strand" of a siRNA molecule refers to a strand that is complementary to the antisense strand or first strand. The antisense and sense strands of a siRNA molecule are hybridized to form a duplex structure. As used herein, a "siRNA duplex" includes a siRNA strand having sufficient complementarity to a section of about 10-50 nucleotides of the mRNA of the gene targeted for silencing and a siRNA strand having sufficient complementarity to form a duplex with the other siRNA strand.

[0399] *Similarity*: As used herein, the term "similarity" refers to the overall relatedness between polymeric molecules, *e.g.* between polynucleotide molecules (*e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of percent similarity of polymeric molecules to one another can be performed in the same manner as a calculation of percent identity, except that calculation of percent similarity takes into account conservative substitutions as is understood in the art.

[0400] *Short interfering RNA or siRNA:* As used herein, the terms "short interfering RNA," "small interfering RNA" or "siRNA" refer to an RNA molecule (or RNA analog) comprising between about 5-60 nucleotides (or nucleotide analogs) which is capable of directing or mediating RNAi. Preferably, a siRNA molecule comprises between about 15-30 nucleotides or nucleotide analogs, such as between about 16-25 nucleotides (or nucleotide analogs), between about 18-23 nucleotides (or nucleotide analogs), between about 19-22 nucleotides (or nucleotide analogs) (e.g., 19, 20, 21 or 22 nucleotides or nucleotide analogs), between about 19-25 nucleotides (or nucleotide analogs), and between about 19-24 nucleotides (or nucleotide analogs). The term "short" siRNA refers to a siRNA comprising 5-23 nucleotides, preferably 21 nucleotides (or nucleotide analogs), for example, 19, 20,

21 or 22 nucleotides. The term "long" siRNA refers to a siRNA comprising 24-60 nucleotides, preferably about 24-25 nucleotides, for example, 23, 24, 25 or 26 nucleotides. Short siRNAs may, in some instances, include fewer than 19 nucleotides, e.g., 16, 17 or 18 nucleotides, or as few as 5 nucleotides, provided that the shorter siRNA retains the ability to mediate RNAi. Likewise, long siRNAs may, in some instances, include more than 26 nucleotides, e.g., 27, 28, 29, 30, 35, 40, 45, 50, 55, or even 60 nucleotides, provided that the longer siRNA retains the ability to mediate RNAi or translational repression absent further processing, e.g., enzymatic processing, to a short siRNA. siRNAs can be single stranded RNA molecules (ss-siRNAs) or double stranded RNA molecules (ds-siRNAs) comprising a sense strand and an antisense strand which hybridized to form a duplex structure called an siRNA duplex.

[0401] *Subject:* As used herein, the term "subject" or "patient" refers to any organism to which a composition in accordance with the disclosure may be administered, *e.g.*, for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and humans) and/or plants.

[0402] *Substantially:* As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0403] *Target Cells:* As used herein, "target cells" or "target tissue" refers to any one or more cells of interest. The cells may be found *in vitro*, *in vivo*, *in situ* or in the tissue or organ of an organism. The organism may be an animal, preferably a mammal, more preferably a human and most preferably a patient.

[0404] *Therapeutic Agent:* The term "therapeutic agent" refers to any agent that, when administered to a subject, has a therapeutic, diagnostic, and/or prophylactic effect and/or elicits a desired biological and/or pharmacological effect.

[0405] *Therapeutically effective amount:* As used herein, the term "therapeutically effective amount" means an amount of an agent to be delivered (*e.g.*, nucleic acid, drug, therapeutic agent, diagnostic agent, prophylactic agent, *etc.*) that is sufficient, when administered to a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is provided in a single dose.

[0406] *Therapeutically effective outcome*: As used herein, the term "therapeutically effective outcome" means an outcome that is sufficient in a subject suffering from or susceptible to an

infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition.

[0407] *Treating*: As used herein, the term "treating" refers to partially or completely alleviating, ameliorating, improving, relieving, delaying onset of, inhibiting progression of, reducing severity of, and/or reducing incidence of one or more symptoms or features of a particular infection, disease, disorder, and/or condition. For example, "treating" cancer may refer to inhibiting survival, growth, and/or spread of a tumor. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition and/or to a subject who exhibits only early signs of a disease, disorder, and/or condition for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[0408] *Conservative amino acid substitution:* As used herein, a "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[0409] *Variant:* As used herein, the term "variant" refers to a polypeptide or polynucleotide that has an amino acid or a nucleotide sequence that is substantially identical, e.g., having at least 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to a reference sequence. In some embodiments, the variant is a functional variant.

[0410] *Functional Variant*: As used herein, the term "functional variant" refers to a polypeptide variant or a polynucleotide variant that has at least one activity of the reference sequence.

[0411] *Insertional Variant*: "Insertional variants" when referring to polypeptides are those with one or more amino acids inserted, e.g., immediately adjacent or subsequent, to a position in an amino acid sequence. "Immediately adjacent" or "immediately subsequent" to an amino acid means connected to either the alpha-carboxy or alpha-amino functional group of the amino acid.

[0412] *Deletional Variant*: "Deletional variants" when referring to polypeptides, are those with one or more amino acids in deleted from a reference protein.

[0413] *Vector:* As used herein, the term "vector" refers to any molecule or moiety which transports, transduces or otherwise acts as a carrier of a heterologous molecule. In some embodiments, vectors may be plasmids. In some embodiments, vectors may be viruses. An AAV particle is an example of a vector. Vectors of the present disclosure may be produced recombinantly and may be

based on and/or may comprise adeno-associated virus (AAV) parent or reference sequences. The heterologous molecule may be a polynucleotide and/or a polypeptide.

[0414] *Viral Genome:* As used herein, the terms "viral genome" or "vector genome" refer to the nucleic acid sequence(s) encapsulated in an AAV particle. A viral genome comprises a nucleic acid sequence with at least one payload region encoding a payload and at least one ITR.

Equivalents and Scope

[0415] The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to certain embodiments, it is apparent that further embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

[0416] The present disclosure is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1. Evaluation of TTM-001 and TTM-002 AAV capsid variants in Diverse Primate Species

[0417] This Example evaluates the tropism and cross-species compatibility of the TTM-001 (SEQ ID NO: 981, comprising the amino acid sequence SPHSKA (SEQ ID NO: 941)) and TTM-002 (SEQ ID NO: 982, comprising the amino acid sequence HDSPHK (SEQ ID NO: 2)) capsid variants in two diverse primate species, marmosets (*Callithrix jacchus*) and African green monkeys (*Chlorocebus sabaeus*), as compared to their tropism in cynomolgus macaques (*Macaca fascicularis*). The cross-species compatibility and tropism of an AAV9 capsid variant comprising the amino acid sequence of SPHKYG (SEQ ID NO: 966) was also investigated in this example. The amino acid sequences of TTM-001 and TTM-002 are provided, e.g., in Table 4.

[0418] To investigate tropism in African green monkeys, AAV particles comprising the TTM-001 capsid variant, the TTM-002 capsid variant, an AAV9 capsid variant comprising SEQ ID NO: 966, or an AAV9 control under the control of a synapsin promoter, were intravenously injected into NHPs (n=2, 3-12 years of age) at a dose of 2E13 vg/kg. After 14-days in life, the brains and tissues (liver, DRG, quadriceps, and heart) of the NHPs were collected and RNA was extracted. Following RNA recovery and RT-PCR amplification, a systematic NGS enrichment analysis was performed to calculate the fold enrichment ratio relative to the AAV9 wild-type control.

[0419] To investigate tropism in marmoset monkeys, AAV particles comprising the TTM-001 capsid variant, the TTM-002 capsid variant, an AAV9 capsid variant comprising SEQ ID NO: 966, or an AAV9 control, were intravenously injected into NHPs (n=2, >10 months of age) at a dose of 2E13 vg/kg (8.75E12 vg/mL). After 28-days in life, the brains and tissues (liver quadriceps, and heart) of the NHPs were collected and RNA was extracted. Following RNA recovery and RT-PCR

amplification, a systematic NGS enrichment analysis was performed to calculate the fold enrichment ratio relative to the AAV9 wild-type control.

[0420] As provided in **Table 20** (African green monkeys) and **Table 21** (marmosets), both the TTM-001 and TTM-002 capsid variants demonstrated increased CNS tropism in diverse primate species. The TTM-001 capsid variant demonstrated a 73.6-fold increase in expression relative to AAV9 in the brain of cynomolgus macaques, a 43.5-fold increase in expression relative to AAV9 in the brain of African green monkeys, and a 703.3-fold increase in expression relative to AAV9 in the brain of marmosets. The TTM-002 capsid variant demonstrated a 62.6-fold increase in expression relative to AAV9 in the brain of cynomolgus macaques, a 13.8-fold increase in expression relative to AAV9 in the brain of African green monkeys, and a 366.6-fold increase in expression relative to AAV9 in the brain of marmosets. Both TTM-001 and TTM-002 led to a significant increase in expression relative to AAV9 in the heart of both African green monkeys and marmosets (**Table 20** and **Table 21**). The AAV9 capsid variant comprising SEQ ID NO: 966 also demonstrated in increase in expression relative to AAV9 in the brain and heart of both African green monkeys and marmosets. Furthermore, TTM-001, TTM-002, and the AAV9 capsid variant comprising SEQ ID NO: 966, also all led to increased expression in the brain of both BALB/c and C57Bl/6 mice, demonstrating an average fold change in expression relative to AAV9 across both species of mice of 63.1, 66.8, and 126.97, respectively.

Table 20. NGS-fold enrichment of TTM-001 (comprises SEQ ID NO: 941), TTM-002 (comprises SEQ ID NO: 2), and an AAV9 capsid variant comprising SEQ ID NO: 966 in African green monkeys

Sequence	SEQ ID NO:	Fold Enrichment relative to AAV9					
		Brain	DRG	Heart	Liver DNA	Liver RNA	Muscle
SPHSKA	941	43.525	1.010	184.789	0.242	1.547	1.715
HDSPHK	2	13.779	0.678	35.991	0.084	0.087	0.144
SPHKYG	966	9.805	0.071	44.865	0.085	0.136	0.234

Table 21. NGS-fold enrichment of TTM-001 (comprises SEQ ID NO: 941), TTM-002 (comprises SEQ ID NO: 2), and an AAV9 capsid variant comprising SEQ ID NO: 966 in marmosets

Sequence	SEQ ID NO:	Fold Enrichment relative to AAV9				
		Brain	Heart	Liver DNA	Liver RNA	Muscle
SPHSKA	941	703.610	48.979	0.268	0.779	0.425
HDSPHK	2	366.625	18.572	0.075	0.276	0.229
SPHKYG	966	150.209	17.232	0.045	0.014	0.146

[0421] Taken together, these data demonstrate that the AAV9 capsid variants of TTM-001 and TTM-002 demonstrated increased CNS tropism relative to the AAV9 control in the CNS across three diverse primate species and two species of mice, providing evidence of strong cross-species capacity. The AAV9 capsid variant comprising the amino acid sequence of SEQ ID NO: 966 also demonstrated strong CNS expression relative to the AAV9 control in two species of NHPs and two species of mice, also showing strong cross-species capacity.

Example 2. Individual Capsid Characterization of TTM-002 in NHPs

[0422] This example describes the transduction level, tropism, ability to cross the blood brain barrier, and overall spatial distribution in the central nervous system (CNS) and peripheral tissues of the AAV capsid variants TTM-002 (SEQ ID NO: 982, comprising the amino acid sequence HDSPHK (SEQ ID NO: 2)) and TTM-001 (SEQ ID NO: 981, comprising the amino acid sequence SPHSKA (SEQ ID NO: 941)), relative to AAV9 following intravenous administration in African green monkeys (*Chlorocebus sabaeus*) and marmosets (*Callithrix jacchus*).

A. Evaluation of TTM-002 in African Green Monkeys (Chlorocebus sabaeus)

[0423] AAV particles were generated with the TTM-002 capsid variant or the AAV9 capsid control which comprised a self-complementary viral genome encoding an histone H2b protein with an HA tag driven by a ubiquitous CBA promoter. The AAV particles comprising the TTM-002 capsid variant or the AAV9 capsid control were administered to the African green monkeys (*Chlorocebus sabaeus*) (n=2) intravenously at a dose of 1e12 VG/kg or 1e13 VG/kg. The in-life period was 28 days and then various CNS and peripheral tissues were collected for measuring transgene mRNA (expression) by RT-qPCR and viral DNA (biodistribution) by ddPCR.

[0424] As shown in **Table 23**, the TTM-002 capsid variant resulted in increased brain biodistribution in all brain regions investigated as compared to AAV9 at both doses tested. The TTM-002 capsid variant also led to increased transgene expression in the brain relative to AAV9 at both doses tested (**Table 24**). In the spinal cord, the TTM-002 capsid variant distributed to the cervical spinal cord and the spinal cord ventral horn at a higher level relative to AAV9 (**Table 23**) and it mediated higher transgene expression than AAV9 in both the full spinal cord and the ventral horn (**Table 24**). The TTM-002 capsid variant exhibited lower biodistribution (**Table 23**) and transgene expression (**Table 24**) in the DRG relative to AAV9, indicating that TTM-002 capsid variant was detargeted in the DRG relative to AAV9. Similar expression and distribution were observed by immunohistochemistry performed on these CNS tissues.

[0425] Distribution and transgene expression was also measured in the peripheral tissues of the liver, heart, and quadriceps. In the liver, TTM-002 capsid variant exhibited lower biodistribution (**Table 23**) and transgene expression (**Table 24**) relative to AAV9, indicating that TTM-002 capsid variant was detargeted in the liver relative to AAV9. In the heart, the TTM-002 capsid variant exhibited comparable levels of biodistribution relative to AAV9 (**Table 23**), but increased transgene expression relative to AAV9 (**Table 24**). In the quadriceps, TTM-002 capsid variant exhibited lower biodistribution (**Table 23**) and lower transgene expression (**Table 24**), relative to AAV9. Similar expression and distribution were observed by immunohistochemistry performed on these peripheral tissues.

Table 23: Quantification of viral genome copies per diploid genome (biodistribution) by ddPCR following intravenous administration of AAV particles comprising a TTM-002 capsid

Tissue	1e12 VG/kg			1e13 VG/kg		
	AAV9 (VG copies/ diploid genome)	**TTM-002** (VG copies/ diploid genome)	**TTM-002 relative to AAV9**	**AAV9** (VG copies/ diploid genome)	**TTM-002** (VG copies/ diploid genome)	**TTM-002 relative to AAV9**
Putamen	0.03	0.37	12.3	0.26	2.4	9.2
Caudate	0.02	0.58	29	0.14	2.1	14.7
Thalamus	0.06	0.21	3.5	0.25	1.0	4
Hippocampus	0.03	0.29	9.7	0.16	1.56	9.8
Substantia Nigra	0.05	0.34	6.8	0.37	1.38	3.7
Motor Cortex	0.03	0.56	19	0.27	2.4	8.9
Frontal Cortex	0.04	0.67	17	0.20	3.6	18
Temporal Cortex	0.03	0.31	10	0.11	2.67	24
Cerebral Cortex	0.008	0.08	10	0.03	0.16	5.3
Dentate Nucleus	0.06	0.10	1.7	0.32	3.21	10
Cervical Spinal Cord	0.03	0.12	4	0.19	0.91	4.8
Thoracic Spinal Cord	0.04	0.03	0.75	0.36	0.38	1.1
Lumbar Spinal Cord	0.04	0.03	0.75	0.29	0.37	1.3
C5 Ventral Horn	0.04	0.25	6.3	0.29	2.2	7.6
L5 Ventral Horn	0.06	0.28	4.7	0.31	1.9	6.1
Cervical DRG	0.07	0.01	-7	0.81	0.36	-2.3
Thoracic DRG	0.06	0.01	-6	1.31	0.43	-3
Lumbar DRG	0.07	0.01	-7	1.31	0.57	-2.3
Liver	9.5	1.2	-7.9	127	7.7	-16.5
Heart	0.6	0.7	1.2	5.4	5.4	1
Quadriceps	0.2	0.06	-3.3	1.7	0.6	-2.8

Table 24: Quantification of transgene mRNA by RT-qPCR following intravenous administration of AAV particles comprising a TTM-002 capsid

Tissue	1e12 VG/kg			1e13 VG/kg		
	AAV9 (transgene mRNA fold over housekeeping gene) (2^{-dCT})	**TTM-002** (transgene mRNA fold over housekeeping gene) (2^{-dCT})	**TTM-002 relative to AAV9**	**AAV9** (transgene mRNA fold over housekeeping gene) (2^{-dCT})	**TTM-002** (transgene mRNA fold over housekeeping gene) (2^{-dCT})	**TTM-002 relative to AAV9**
Putamen	0.02	0.3	15	0.09	4.22	47
Caudate	0.02	0.8	40	0.11	4.29	39
Thalamus	0.04	0.4	10	0.4	5.8	14.5
Hippocampus	0.02	0.4	20	0.1	4.3	43
Substantia Nigra	0.1	1.2	12	0.3	11.6	39
Motor Cortex	0.08	5.00	63	0.36	21.8	61
Frontal Cortex	0.04	3.1	78	0.3	27.7	92

Temporal Cortex	0.02	0.8	40	0.1	26.9	27
Cerebral Cortex	0.04	1.1	28	0.2	17.4	87
Dentate Nucleus	0.3	0.9	3	1.8	42.0	23
Cervical Spinal Cord	0.2	2.0	10	0.8	20.2	25
Thoracic Spinal Cord	0.13	0.25	1.9	0.7	4.8	6.9
Lumbar Spinal Cord	0.4	0.5	1.3	2.2	9.2	4.2
C5 Ventral Horn	0.2	1.4	7	1.7	33	19
L5 Ventral Horn	1.1	3.4	3.1	12.4	102	8.2
Cervical DRG	3.6	1.2	-3	63.1	15.9	-4
Thoracic DRG	1.8	1.3	-1.4	43.9	15.7	-2.8
Lumbar DRG	1.9	1.0	-1.9	34.9	27.6	-1.3
Liver	0.88	0.25	-3.5	2.2	0.97	-2.3
Heart	8.7	42	4.8	110	363	3.3
Quadriceps	9.7	1.1	-8.3	59	21	-2.8

[0426] Taken together, these data demonstrate that TTM-002 is an enhanced CNS tropic capsid in NHPs (African green monkeys) that can infect non-neuronal cells. TTM-002 was also detargeted in the DRG and liver relative to AAV9, but showed increased transgene expression in the heart relative to AAV9. Additionally, the TTM-002 capsid variant was able to successfully penetrate the blood brain barrier following intravenous injection.

B. Evaluation of TTM-001 and TTM-002 in Marmosets (Callithrix jacchus)

[0427] AAV particles were generated with the TTM-002 capsid variant, the TTM-001 capsid variant, or the AAV9 capsid control which comprised a self-complementary viral genome encoding a histone H2b protein with an MYC tag (TTM-002 capsid variant), His tag (TTM-001 capsid variant), or HA tag (AAV9 control capsid) driven by a ubiquitous CAG promoter. The AAV particles comprising the TTM-002 capsid variant, the TTM-001 capsid variant, or the AAV9 capsid control were administered to the marmosets (*Callithrix jacchus*) (n=3) intravenously in a single solution, at the doses indicated in **Table 34**. The in-life period was 28 days and then various CNS and peripheral tissues were collected for measuring transgene mRNA (expression) by RT-qPCR, protein expression by IHC, and viral DNA (biodistribution) by ddPCR. Data were then normalized to the dose of each viral vector in the dosing solution.

Table 34. Titer of the AAV particles comprising the various capsids in solution dosed in marmosets

Capsid Variant	Actual Titer Dosed	Ratio of Capsid Variant to AAV9
TTM-001	1.44×10^{11} vg/mL	0.34
AAV9	4.00×10^{11} vg/mL	1.0
TTM-002	4.17×10^{11} vg/mL	1.0

[0428] As shown in **Table 35**, both the TTM-001 and TTM-002 capsid variants demonstrated increased biodistribution in the caudate and motor cortex in the brain of the marmosets relative to the AAV9 control. The TTM-001 and TTM-002 capsid variants also led to increased transgene expression (**Table 36**) in the caudate and motor cortex in the brain of the marmosets. In fact, biodistribution and transgene expression were increased over 100-400 fold for both TTM-001 and TTM-002 in the brain relative to AAV9. Similar expression and distribution was observed by immunohistochemistry. More specifically, staining for TTM-001 and TTM-002 was detected in the mid-brain, caudate, putamen, thalamus, and cerebellum, and this staining was increased for both capsid variants in each of these brain tissues relative to AAV9. Staining for TTM-001 and TTM-002 was also observed in the molecular and granule layer of the cerebellum.

[0429] Distribution and transgene expression was also measured in the peripheral tissues of the liver, heart, and quadriceps. In the liver, the TTM-002 capsid variant exhibited lower biodistribution (**Table 35**) and transgene expression (**Table 36**) relative to AAV9, indicating that the TTM-002 capsid variant was detargeted in the liver relative to AAV9 in marmosets. The TTM-001 capsid variant demonstrated comparable biodistribution and transgene expression in the liver (**Table 35** and **Table 36**) as well as comparable transgene expression in the heart and muscle (**Table 36**) relative to AAV9. Both TTM-001 and TTM-002 led to decreased biodistribution (**Table 35**) relative to AAV9 in the heart and muscle, and TTM-002 also resulted in lower transgene expression in the heart and muscle relative to AAV9 (**Table 36**).

Table 35. Quantification of viral genome copies per diploid genome (biodistribution) by ddPCR following intravenous administration of AAV particles comprising a TTM-001 capsid or a TTM-002 capsid normalized to the actual titer of the viral vector in the dosing solution (vg/dg = viral genome copies/ diploid genome)

Capsid	Tissue									
	Caudate		Motor Cortex		Heart		Muscle		Liver	
	vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9	vg/dg	vg/dg relative to AAV9
TTM-001	1.67	142.70	2.69	124.06	0.28	0.53	0.08	0.39	14.86	0.99
TTM-002	3.55	294.36	5.80	264.86	0.33	0.69	0.08	0.32	6.92	0.49
AAV9	0.01	1.00	0.02	1.00	0.48	1.00	0.23	1.00	13.79	1.00

Table 36. Quantification of transgene mRNA by RT-qPCR following intravenous administration of AAV particles comprising a TTM-001 capsid or a TTM-002 capsid normalized to the actual titer of the viral vector in the dosing solution (mRNA = transgene mRNA fold over housekeeping gene; rel. to AAV9= transgene mRNA fold over housekeeping gene relative to AAV9)

Capsid	Tissue									
	Caudate		Motor Cortex		Heart		Muscle		Liver	
	mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9	mRNA	rel. to AAV9
TTM-001	17.56	594.71	27.80	586.23	16.05	1.40	0.26	1.19	3.23	1.93

TTM-002	14.21	479.39	19.73	410.40	2.78	0.27	0.06	0.46	0.62	0.36
AAV9	0.03	1.00	0.05	1.00	12.67	1.00	0.15	1.00	1.85	1.00

[0430] These data in marmosets for TTM-002 were similar to those observed in African green monkeys, further demonstrating cross-species compatibility of the TTM-002 capsid variant.

[0431] Taken together, these data demonstrate that TTM-001 and TTM-002 are enhanced CNS tropic capsids in marmosets. TTM-002 was also detargeted in the liver, heart, and muscle relative to AAV9 in marmosets, where TTM-001 demonstrated comparable biodistribution and/or transgene expression in the liver, heart, and muscle compared to AAV9. Additionally, the TTM-001 and TTM-002 capsid variants were able to successfully penetrate the blood brain barrier following intravenous injection.

Example 3. Identification of a receptor for TTM-001 and TTM-002 capsid variants

[0432] This example investigates the tropism and receptor of the TTM-001 (SEQ ID NO: 981, comprising the amino acid sequence SPHSKA (SEQ ID NO: 941)) and TTM-002 (SEQ ID NO: 982, comprising the amino acid sequence HDSPHK (SEQ ID NO: 2)) capsid variants for crossing the blood brain barrier. Without wishing to be bound by theory, it is believed that identification of a receptor of these AAV capsid variants provides a better understand of translatability of these variants to different species, as well as the mechanism used for crossing the blood brain barrier that results in an increase in CNS transduction relative to AAV9.

A. Binding of TTM-001 and TTM-002 capsid variants to N-linked galactose

[0433] Primary glycan receptors have been identified for various AAV serotypes, including AAV9 which binds N-linked galactose. In order to investigate the ability of TTM-001 and TTM-002 AAV9 variants to retain this natural glycan binding, HeLa cells were treated with increasing concentrations of Neuraminidase (0, 5, 50, 500, and 100 mU/mL), which cleaves N-sialic acid and exposes N-galactose. The treated cells were then transduced with AAV particles comprising the TTM-001 capsid variant (AAV_TTM-001), the TTM-002 capsid variant (AAV_TTM-002), or an AAV9 control (AAV_AAV9cntl), transduction was measured by quantification of Luc2 activity (RLU), and data was normalized relative to the no neuraminidase control. As shown in **Table 25**, enzymatic removal of N-sialic acid and exposure of N-galactose on HeLa cells, resulted in a dose dependent increase, more specifically a 9- to 14-fold increase, in transduction by AAV particles comprising the TTM-001 capsid variant and AAV particles comprising the TTM-002 capsid variant. This was analogous to what was observed with the AAV9 control (**Table 25**). These data demonstrate that the TTM-001 and TTM-002 AAV9 capsid variants retained the natural binding affinity to terminal N-linked galactose observed with AAV9 wild-type.

Table 25. Quantification of HeLa cell transduction post-neuraminidase treatment and transduction with AAV_TTM-001 particles, AAV_TTM-002 particles, or AAV_AAV9cntrl

particles. Data measured as fold change in Luc2 activity (RLU) relative to the no neuraminidase control

Capsid	Concentration of Neuraminidase			
	5 mU/mL	50 mU/mL	500 mU/mL	1000 mU/mL
AAV9 (control)	2.7	6.2	11.1	10.4
TTM-001	5.2	6.7	8.2	9.1
TTM-002	9.6	12.5	13.9	13.7

B. Receptor Identification

[0434] A cell binding array assay was then used to identify a receptor for the TTM-001 and TTM-002 capsid variants. Briefly, a library of over 5,500 cDNAs was overexpressed in human cells. Cells were contacted with a test ligand, e.g., AAV viral particles comprising a TTM-001 capsid variant or an AAV9 control capsid, which was applied to the array. Binding of the TTM-001 capsid variant or the AAV9 control capsid to the cells was detected using an anti-AAV9 antibody followed by a labeled anti-IgG detection antibody. A comparison of the proteins contacted using AAV particles comprising a wild type AAV9 control capsid and AAV particles comprising a TTM-001 capsid variant revealed a unique interaction with the TTM-001 capsid variant but not the AAV9 wild-type control capsid. This interacting protein was identified as the GPI-anchored protein, alkaline phosphatase issue-nonspecific isozyme (NM_000478.4, which is incorporated by reference herein) (ALPL). ALPL is part of a family of membrane-bound glycoproteins that hydrolyze monophosphate esters at a high pH (see, e.g., Weiss et al., Isolation and characterization of a cDNA encoding a human liver/bone/kidney-type alkaline phosphatase. Proc. Natl. Acad. Sci., 83: 7182-7186 (1986), the contents of which are hereby incorporated by reference in their entirety).

[0435] ALPL is highly conserved across humans, mice, and cynomolgus macaques (*Macaca fascicularis*) when compared by sequence alignment (**Table 26**). Additionally, in humans ALPL is expressed on endothelial cells and neurons, and at a low level on astrocytes. The highest level of ALPL expression in human is on endothelial cells. In mice, ALPL is more highly expressed on astrocytes, oligodendrocyte progenitor cells (OPCs), and to a lesser extent on endothelial cells.

Table 26. Identity and similarity of ALPL receptor between different, species

	Percent Identity	Percent Similarity
Human to cynomolgus macaque	97.33%	98.5%
Human to mouse	89.89%	94.47%
Cynomolgus macaques to mouse	90.46%	95.23%

[0436] Furthermore, when mice were treated intravenously with AAV particles comprising the TTM-002 capsid variant expressing a payload, payload expression as measured by RNA-seq was the highest in a subset of endothelial cells (**FIG. 1A**). This same subset of endothelial cells also showed high expression of ALPL by RNA-seq (**FIG. 1B**). These data indicated a correlation between expression of ALPL and the TTM-002 tropism in mice.

[0437] Taken together, these data indicate that the TTM-001 and TTM-002 capsid variants are capable of binding ALPL, which could serve as a receptor for crossing the blood brain barrier and CNS transduction.

C. Characterization of interaction of the TTM-001 and/or TTM-002 with ALPL

[0438] In order to further characterize the interaction between the TTM-001 and TTM-002 capsid variants and the ALPL protein, it was investigated whether increased expression of the ALPL protein resulted in increased transduction of AAV particles comprising the TTM-001 or TTM-002 capsid variant. Briefly, a transduction assay was performed in that HEK 293T cells were transfected via calcium phosphate transfection with a plasmid expressing ALPL, an AAVR positive control, or a pCMV6 negative control (250 ng or 500 ng of plasmid). AAVR is a universal AAV entry factor involved in AAV transduction. At 24 hours post-transfection, the HEK 293T cells expressing the ALPL protein or other controls were transduced with an AAV particle comprising the TTM-001 capsid variant, the TTM-002 capsid variant, another AAV capsid variant (TTD-001), or an AAV9 control capsid protein, expressing a GFP payload. At 24-hours post-transduction, GFP expression and luciferase activity were measured to quantify and observe AAV cellular transduction. By immunofluorescence microscopy, expression of the ALPL protein resulted in a significant increase in the transduction of AAV particles comprising the TTM-002 capsid variant compared to particles comprising the AAV9 wild-type control capsid. Additionally, the increase in transduction of the AAV particles comprising the TTM-002 capsid variant was specific to ALPL expression, as expression of the AAVR control did not result in the same increase in transduction of AAV particles comprising the TTM-002 capsid variant. As summarized in **Table 27**, expression of ALPL led to a 35 and 45-fold increase in transduction the TTM-001 and TTM-002 AAV9 capsid variants, respectively, when measured by a luciferase assay. Transduction of AAV9 wild-type control as well as the AAV9 capsid variant TTD-001 was not affected by expression of ALPL, indicating the specific role of ALPL in transduction of TTM-001 and TTM-002. TTD-001 is an AAV9 capsid variant comprising a loop VIII modification, and the sequence and capsid characterized can be found in WO 2021/230987, the contents of which are hereby incorporated by reference in their entirety). **Table 28** provides the results of a second experiment performed as described above, where HEK 293T cells expressing the ALPL protein or the other controls were transduced with AAV particles comprising the TTM-002 capsid variant or one of three AAV9 capsid variants also comprising a modification in loop IV: TTM-006 (SEQ ID NO: 39), TTM-018 (SEQ ID NO: 51), and TTM-019 (SEQ ID NO: 52). The TTM-002, TTM-006, TTM-018, and TTM-019 capsid variants all comprised the SPH motif immediately subsequent to position 455, numbered relative to SEQ ID NO: 138 and a positive residue in one of the next three residues subsequent to the SPH motif. The TTM-002, TTM-006, TTM-018, and TTM-019 capsid variants all resulted in an increase in transduction in cells expressing ALPL, which was not observed with the AAV9 control (**Table 28**). **Table 29** provides the results of a third experiment performed as described above, where HEK 293T cells expressing the ALPL protein or the other

controls were transduced with AAV particles comprising the TTM-001 capsid variant, TTM-002 capsid variant, or the TTM-003 capsid variant (SEQ ID NO: 36) which comprised the SPH motif immediately subsequent to position 455, numbered relative to SEQ ID NO: 138 and a positive residue in one of the next three residues subsequent to the SPH motif. The TTM-001, TTM-002, and TTM-003 capsids all resulted in an increase in transduction in cells expressing ALPL, which was not observed in the AAV9 control (**Table 29**). The increase in transduction was greater in cells transduced with TTM-001 or TTM-002 compared to TTM-003 (**Table 29**).

Table 27. Transduction of TTM-001 and TTM-002 capsid variants as measured by luciferase assay relative to the AAV9 control and AAV variant TTD-001 with loop VIII modification (data shown as fold change relative to the pCMV6 transfected negative control cells)

	Luciferase Activity (fold change relative to pCMV6 transfected cells)		
Capsid	**pCMV6 transfected cells**	**AAVR transfected cells**	**ALPL transfected cells**
AAV9	1	4.3	1.5
TTD-001	1	22	2
TTM-001	1	2.4	35
TTM-002	1	4.1	44

Table 28. Transduction of TTM-002, TTM-006, TTM-018, and TTM-019 capsid variants as measured by luciferase assay relative to the AAV9 control (data shown as fold change relative to the pCMV6 transfected negative control cells)

	Luciferase Activity (fold change relative to pCMV6 transfected cells)		
Capsid	**pCMV6 transfected cells**	**AAVR transfected cells**	**ALPL transfected cells**
AAV9	1	14	0.9
TTM-002	1	24	166
TTM-006	1	25	91
TTM-018	1	25	90
TTM-019	1	28	88

Table 29. Transduction of TTM-001, TTM-002, and TTM-003 capsid variants as measured by luciferase assay relative to the AAV9 control (data shown as fold change relative to the pCMV6 transfected cells)

AAV9			**TTM-001**			**TTM-002**			**TTM-003**		
pCMV6	AAVR	ALPL	pCMV6	AAVR	ALPL	pCMV6	AAVR	ALPL	pCMV6	AAVR	ALPL
1.01	4.04	1.70	0.94	3.22	22.37	1.19	7.04	32.71	0.85	1.73	6.10
0.83	4.08	1.61	1.01	3.25	28.45	0.93	5.32	36.63	0.72	1.24	5.98
1.15	3.69	2.29	1.05	3.31	26.93	0.88	6.49	37.38	1.43	1.33	7.50

[0439] Binding and internalization of AAV capsid variants comprising the TTM-001 capsid variant, the TTM-002 capsid variant, or the AAV9 control capsid was also investigated in cells engineered to express ALPL. HEK 293T cells were transfected via calcium phosphate transfection with a plasmid expressing ALPL, an AAVR positive control, or a pCMV6 negative control. At 24 hours post-transfection, the HEK 293T cells expressing the ALPL receptor were incubated with an AAV particle comprising the TTM-001 capsid variant, the TTM-002 capsid variant, or an AAV9

control capsid protein, expressing a GFP payload. At 2 or 3-hours post-incubation, cells were washed to remove unbound AAV particles and DNA was extracted to quantify viral genomes. As shown in **Table 29**, expression of ALPL led to a 3-fold and 6-fold increase in binding/internalization by TTM-001 and TTM-002, respectively. This effect was specific to TTM-001 and TTM-002, as the binding/internalization of the wild-type AAV9 control was unaffected by ALPL expression.

Table 29. Relative viral gene expression ($2^{-\Delta\Delta CT}$) of cells transfected with a plasmid expressing pCMV6 control, an AAVR control, or ALPL and subsequently transduced with AAV particles comprising the TTM-001 capsid variant, TTM-002 capsid variant, or AAV9 control

	Fold change in viral gene expression ($2^{-\Delta\Delta CT}$) relative to pCMV6 transfected cells		
Capsid	pCMV6 transfected cells	AAVR transfected cells	ALPL transfected cells
AAV9	1	7.04	1.06
TTM-001	1	6.64	3.16
TTM-002	1	3.2	5.92

[0440] Three isoforms of ALPL exist, isoform 1 (Alkaline phosphatase, placental-like 2 (ALPPL2), NM_031313, which is incorporated by reference herein), isoform 2 (alkaline phosphatase, placental (ALPP), NM_001632, which is incorporated by reference herein), and isoform 3 (alkaline phosphatase, intestinal (ALPLI), NM_001631, which is incorporated by reference herein), that can also be expressed on cell surfaces via a GPI-anchor. Isoform 1 is 56.25% identical and 72.54% similar (gaps: 4.17%) to ALPL, isoform 2 is 54.96% identical and 71.37% similar (gaps: 2.29%) to ALPL, and isoform 3 is 55.98% identical and 72.11% similar (gaps: 3.04%) to ALPL. The transduction assay described above was repeated with the three isoforms. HEK 293T cells were transfected via calcium phosphate transfection with a plasmid expressing ALPL, isoform 1 of ALPL, isoform 2 of ALPL, isoform 3 of ALPL, an AAVR positive control, or a pCMV6 negative control. At 24 hours post-transfection, the HEK 293T cells expressing the ALPL receptor were transduced with an AAV particle comprising the TTM-001 capsid variant or the TTM-002 capsid variant, expressing a Luc2-GFP payload. At 24-hours post-transduction, luciferase activity (RLU) was measured to quantify AAV cellular transduction. As shown in **Table 30**, the increase in transduction observed for TTM-001 and TTM-002 when cells express ALPL did not occur in cells expressing isoform 1, 2, or 3. This demonstrates that the significant increase in TTM-001 and TTM-002 transduction is a specific function of ALPL.

Table 30. Transduction of TTM-001 and TTM-002 capsid variants as measured by luciferase assay (RLU) in cells expressing ALPL or isoforms thereof

	Luciferase Activity (RLU)					
Capsid	pCMV6 transfected cells	AAVR transfected cells	ALPL transfected cells	Isoform 1 transfected cells	Isoform 2 transfected cells	Isoform 3 transfected cells
TTM-001	5,212	13,981	93,268	4,072	7,456	3,300
TTM-002	2,894	11,261	46,182	1,114	3,465	2,074

[0441] Endogenous ALPL was also removed from the surface of HeLa cells by treatment with increasing concentrations phosphatidylinositol-specific phospholipase C (PI/PLC), which cleaves GPI anchored proteins (0, 1, 3, 6, or 10 U/mL), for 1.5 hours at 37°C. Following PI/PLC treatment, cells were incubated with 1E4 VG/cell for three hours of AAV particles comprising the TTM-002 capsid variant or AAV particles comprising an AAV9 control capsid, cells were then washed to remove free virus, and luciferase activity was measured 24 hours post-transduction (RLU). As shown in **Table 31**, treatment with PI/PLC and removal of the GPI-anchored proteins, significantly decreased transduction by the TTM-002 capsid variant, indicating that increased transduction by TTM-002 in HeLa cells is dependent on a GPI-anchored protein.

Table 31. Transduction of TTM-002 capsid variant or AAV9 control in HeLa as measured by luciferase assay (RLU) following treatment with PI/PLC

	Concentration of PI/PLC				
Capsid	**0 U/mL**	**1 U/mL**	**3 U/mL**	**6 U/mL**	**10 U/mL**
AAV9	239.3	193.7	222	207.7	212
TTM-002	1731	474	441	396.7	239.3

[0442] To determine if deletion of the endoplasmic reticulum (ER) localization signal of ALPL affected transduction of the TTM-001 and TTM-002 capsid variants, HEK 293T cells were transfected via calcium phosphate transfection with a plasmid expressing ALPL, ALP with a deletion of the ER localization signal (ALPL transcript variant 2 that lacks the ER signal (NM_001127501, which is incorporated by reference herein)), or a pCMV6 negative control. At 24 hours post-transfection, the HEK 293T cells expressing the ALPL receptor were transduced with an AAV particle comprising the TTM-001 capsid variant, the TTM-002 capsid variant, or an AAV9 capsid control, expressing a GFP payload. At 24-hours post-transduction, luciferase activity (RLU) was measured to quantify AAV cellular transduction. Data was normalized to fold change in luciferase activity (RLU) compared to the pCMV6 control. As shown in **Table 32**, the increase in transduction observed for TTM-001 and TTM-002 when cells express ALPL did not occur in cells expressing ALPL comprising a deleted ER localization signal, and therefore did not express ALPL on the surface of the cells. Similar results were observed by immunofluorescence microscopy staining for GFP expression, as no GFP staining was observed in cells transfected with the ALPL mutant comprising a deletion of the ER localization signal that were transduced with AAV particles comprising the TTM-001 and TTM-002 capsid variants. These data demonstrate that the ER localization signal may play an important role in effect ALPL has on the transduction of the TTM-001 and TTM-002 capsid variants.

Table 32. Transduction of TTM-001 and TTM-002 capsid variants as measured by luciferase assay (RLU) in cells expressing ALPL

Capsid	Fold change in luciferase activity relative to the pCMV6 control cells		
	Cells transfected with pCMV6	Cells transfected with ALPL	Cells transfected with ALPL comprising a deletion of the ER localization signal
AAV9	1	1.4	1.7
TTM-001	1	22	2.7
TTM-002	1	24	2.7

[0443] To determine if the TTM-001 and TTM-002 capsid variants could bind to both the human ALPL protein (NM_000478.6, which is incorporated by reference herein) and the mouse ALPL ortholog (NM_001287172.1, which is incorporated by reference herein), HEK 293T cells were transfected via calcium phosphate transfection with a plasmid expressing human ALPL, the murine ortholog of ALPL, or a pCMV6 negative control. At 24 hours post-transfection, the HEK 293T cells expressing the ALPL receptor were transduced with an AAV particle comprising the TTM-001 capsid variant, the TTM-002 capsid variant, or an AAV9 control capsid protein, expressing a Luc2-GFP payload. At 24-hours post-transduction, luciferase activity (RLU) was measured to quantify AAV cellular transduction. As shown in **Table 33**, the increase in transduction observed for TTM-001 and TTM-002 when cells express human ALPL was also observed in cells expressing the murine ALPL ortholog. These luciferase results were also confirmed by immunofluorescence microscopy staining for GFP. These data indicate that the murine ALPL protein is also a receptor for the TTM-001 and TTM-002 capsid variants.

Table 33. Transduction of TTM-001 and TTM-002 capsid variants as measured by luciferase assay (RLU) in cells expressing ALPL

Capsid	Fold change in luciferase activity relative to the pCMV6 control cells		
	Cells transfected with pCMV6	Cells transfected with human ALPL protein	Cells transfected with murine ALPL protein
AAV9	1	1	2
TTM-001	1	22	18
TTM-002	1	24	24

[0444] Direct binding and specific interaction of the TTM-002 capsid variant and the AAV9 capsid control to ALPL was measured by Surface Plasmon Resonance (SPR) on Biacore 8K instrument. His-tagged ALPL was first captured on a CM5 sensor chip pre-immobilized with anti-His antibody by passing 5 µg/ml of ALPL for 240 seconds. AAV9 or TTM-002 and buffer were then passed over ALPL to monitor rates of association and dissociation, respectively. The concentration of AAV used was from 0.0625 to 1 nM (e.g., 0.0625 nM, 0.125 nM, 0.25 nM, 0.5 nM, and 1 nM; **FIGs. 2A-2B**) or from 15.625 to 250 nM (e.g., 15.625 nM, 32.25 nM, 62.5 nM, 125 nM, and 250 nM; **FIGs. 2C-2D**) and association/dissociation rates were monitored for 120 seconds. The surface was regenerated using two pulses of 10 mM glycine pH 1.7 for 30 seconds. A flow rate of 30 µl/min was

used for all steps and the running buffer used was PBS-P+. As shown in **FIG 2A** and **FIG. 2C**, TTM-002 was capable of directly and specifically binding to ALPL in a dose dependent manner, where as AAV9 showed no binding (**FIG. 2B** and **FIG. 2D**). The dissociation constant (K_D) was quantified for the TTM-002 capsid variant binding to ALPL, and was determined to be approximately 32 nM.

[0445] Additionally, siRNAs were used to knockdown endogenous levels of ALPL in HeLa cells. HeLa cells were transfected with one of two siRNAs targeting ALPL, both siRNAs targeting ALPL, or a non-ALPL targeting siRNA control using lipofectamine 2000 (5pmol of the siRNA per well of 96-well plate). At 48-hours post transfection, the cells were transduced with 1E4 VG/cell of AAV particles comprising a TTD-002 capsid variant or an AAV9 control capsid and a viral genome encoding a Luc2-GFP payload. At 24-hours post transduction, luciferase activity (RLU) was measured to quantify AAV cellular transduction (**FIG 3**). siRNA mediated knockdown of ALPL led to a 60% reduction in TTM-002 transduction, indicating that knockdown of endogenous ALPL expression inhibits TTM-002 transduction.

D. Conclusions

[0446] Taken together, these data demonstrate the ALPL is a likely surface receptor for the TTM-001 and TTM-002 capsid variants, as overexpression led to an increase in TTM-001 and TTM-002 transduction as well as cell binding/internalization, which was specific for ALPL. Enzymatic removal of ALPL from the cell surface, mutating the ER localization signal of ALPL, or knockdown of the ALPL receptor by siRNA, also reduced TTM-002 transduction. Without wishing to be bound by theory, it is believed in some embodiments, that the binding of TTM-001 and TTM-002 to ALPL is part of the mechanism leading to increased crossing of the blood brain barrier relative to the AAV9 control. The highly conserved nature of the ALPL receptor protein across species is predictive of cross-species compatibility of the TTM-001 and TTM-002 capsid variants.

Example 4. *In vitro* screen of TRACER AAV libraries in cells expressing a GPI anchored protein

[0447] A library of AAV capsid variants was generated using a sliding window approach, where 6 amino acid sequences were inserted into different positions across loop IV of AAV9, including immediately subsequent to positions 453 and 455, relative to a reference sequence numbered according to SEQ ID NO: 138, 981, or 982. This library was then transduced into HEK293T cells that over-expressed the GPI-anchored protein, alkaline phosphatase (ALPL) and wild-type HEK293T cells. RNA was extracted from the transduced cells. Following RNA recovery and RT-PCR amplification, a systematic NGS enrichment analysis was performed. The peptide sequence comprised within the capsid variants were identified and the average fold-change (of NGS reads, counts per million) was calculated for each variant relative to virus input in the HEK293T cells that over-expressed ALPL and the wild-type HEK293T cells.

[0448] **Table 37** provides the fold-change data for the capsid variants that comprised a peptide insert present immediately subsequent to position 455 (e.g., present at positions 456-461), numbered according to SEQ ID NO: 981, that comprised an SPH motif within the insert sequence and demonstrated a fold-change greater than 1 in cell over-expressing ALPL. **Table 38** provides the fold-change data for the capsid variants that comprised a peptide insert present immediately subsequent to position 453 (e.g., present at positions 454-459), numbered according to SEQ ID NO: 982, that comprised an SPH motif within the insert sequence, and demonstrated a fold-change greater than 1 in cell over-expressing ALPL. A high average fold-change in the HEK293T cells expressing ALPL and a low fold-change in the wild-type HEK293T cells, is indicative of a capsid variant that is capable of transducing the cells over-expressing ALPL to a higher degree than wild-type cells.

Table 37. NGS fold-change of AAV capsid variants that comprised a peptide insert present immediately subsequent to position 455, numbered according to SEQ ID NO: 981, in HEK293T cells expressing ALPL and wild-type HEK293T cells relative to virus input

Sequence	Fold-Change in WT cells	Fold-Change in ALPL expressing-cells	SEQ ID NO:	Sequence	Fold-Change in WT cells	Fold-Change in ALPL expressing-cells	SEQ ID NO:
SPHRGP	0.000	292.021	1100	SPHPIR	0.000	17.993	1265
SPHRGG	0.068	259.306	1101	SPHHMR	0.000	17.166	1266
SPHHGR	0.000	235.145	1102	SPHMQR	0.000	16.976	1267
SPHARM	0.000	223.853	947	SPHSAY	0.000	16.928	1268
SPHGRS	0.000	218.549	1103	SPHVSK	0.000	16.540	1269
SPHAMR	0.000	206.291	1104	SPHMRS	0.000	16.055	1270
SPHAQR	5.298	204.015	1105	SPHERF	2.752	15.861	1271
SPHRAT	4.455	176.003	1106	SPHLRG	0.000	14.958	1272
SPHRGI	0.000	192.170	1107	SPHHYA	0.000	12.190	1273
SPHARV	1.775	190.700	1108	SPHAGP	0.000	11.783	1274
SPHQSR	0.000	185.035	1109	SPHPKA	4.527	8.013	1275
SPHARG	0.094	180.773	1110	SPHAHL	0.000	10.191	1276
SPHRGL	2.322	177.671	1111	SPHEKW	0.000	7.493	1277
SPHRGM	0.050	176.978	1112	SPHMPR	0.000	6.669	1278
SPHQVR	0.000	174.139	1113	SPHLVR	0.000	5.028	1279
SPHKSL	0.000	170.164	4740	SPHPRT	0.000	4.882	1280
SPHRSL	0.256	166.362	1114	SPHGPA	0.000	4.816	1281
SPHGRN	0.000	162.003	1115	SPHLPR	0.036	3.364	1282
SPHVRQ	3.612	157.188	1116	SPHDRG	0.000	2.707	1283
SPHQRS	5.186	121.080	1117	NSPHRS	0.000	190.560	1284
SPHGFR	0.000	156.137	1118	TSPHRG	0.000	186.404	1285
SPHRTA	1.259	128.814	1119	GSPHKR	0.000	160.607	1286
SPHGKW	0.000	155.225	1120	NSPHKQ	0.000	151.475	1287
SPHSRL	1.130	152.841	1121	GSPHRH	0.000	134.659	1288
SPHTRP	0.000	147.875	1122	GSPHRS	0.000	130.695	1289
SPHGRG	1.389	137.093	1123	NSPHKW	0.254	127.620	1290
SPHSRM	3.763	169.054	1124	SSPHSR	0.000	114.961	1291
SPHRAA	1.468	142.982	1125	NSPHSR	0.000	113.377	1292
SPHGGK	0.080	138.950	1126	GSPHIK	0.000	112.012	1293
SPHGRL	1.565	138.697	1127	QSPHRS	0.545	111.174	1294

SPHSRP	0.027	136.630	1128	GSPHRG	0.000	101.043	1295
SPHANR	9.620	182.281	1129	SSPHKV	0.000	98.767	1296
SPHAVR	0.000	133.502	1130	GSPHSR	0.000	98.213	1297
SPHRYT	0.000	131.562	1131	SSPHRG	0.134	93.804	1298
SPHRSF	0.000	129.885	1132	GSPHMK	0.713	93.219	1299
SPHRAG	0.020	127.161	1133	GSPHGR	0.000	93.061	1300
SPHRAQ	0.000	126.697	1134	QSPHSR	0.000	92.888	1301
SPHSPR	6.622	84.871	1135	ASPHRS	0.080	89.415	1302
SPHRAP	0.000	126.073	1136	SSPHRI	0.000	79.804	1303
SPHPSR	0.067	125.119	1137	SSPHVK	0.000	75.668	1304
SPHRGS	0.000	124.646	1138	GSPHRL	0.000	72.850	1305
SPHRMV	3.395	187.734	1139	TSPHHR	0.615	67.422	1306
SPHARL	4.750	122.713	1140	SSPHNR	0.000	65.742	1307
SPHRTS	0.489	121.608	1141	ASPHRG	0.076	64.798	1308
SPHHKP	0.000	120.939	1142	ASPHGR	0.000	54.613	1309
SPHGKF	0.000	120.579	1143	GSPHIR	0.000	53.631	1310
SPHARP	0.122	119.047	1144	MSPHSR	0.000	52.299	1311
SPHARQ	0.000	118.401	1145	HSPHRN	0.000	51.076	1312
SPHSGR	0.511	116.488	1146	TSPHKK	0.000	50.887	1313
SPHTYR	1.168	109.956	1147	NSPHRT	0.548	50.774	1314
SPHHAR	0.000	114.527	1148	GSPHRI	0.000	48.035	1315
SPHRVS	0.000	113.346	1149	SSPHRA	4.299	81.033	1316
SPHGNR	0.080	112.821	1150	ASPHQR	0.827	34.628	1317
SPHSRQ	5.808	168.226	1151	GSPHAH	0.000	46.495	1318
SPHGRV	0.000	109.730	1152	ESPHRK	0.876	46.368	1319
SPHVRG	0.000	109.327	973	VSPHRS	0.000	46.145	1320
SPHSIR	0.168	108.586	1153	GSPHRN	0.000	44.772	1321
SPHRHT	4.977	74.311	1154	YSPHRS	0.000	44.057	1322
SPHPRP	0.000	107.223	1155	ASPHKI	1.393	90.525	1323
SPHRPM	0.000	104.274	1156	SSPHFR	4.963	29.243	1324
SPHRQP	0.000	102.899	1157	QSPHNR	0.000	37.781	1325
SPHSRH	0.000	102.537	1158	VSPHRG	0.000	36.826	1326
SPHGPR	10.563	119.049	1159	MSPHRG	2.438	30.486	1327
SPHRLL	0.000	101.884	1160	SSPHMR	1.852	35.914	1328
SPHMHR	0.000	101.583	1161	ASPHAK	0.036	35.250	1329
SPHKVR	0.000	101.257	1162	LSPHSR	0.186	34.183	1330
SPHRAV	0.000	100.726	1163	HSPHRS	3.169	32.967	1331
SPHTRT	0.062	98.189	1164	QSPHKG	0.000	33.440	1332
SPHGYR	8.187	113.395	1165	QSPHRA	0.375	32.317	1333
SPHAKS	0.000	98.142	1166	ASPHRV	1.863	27.187	1334
SPHQTK	0.000	96.538	1167	VSPHRL	0.023	31.413	1335
SPHATR	0.000	94.840	1168	ASPHNR	2.438	42.454	1336
SPHRTV	0.000	93.448	1169	ASPHRA	3.251	30.750	1337
SPHVSR	0.000	92.032	1170	SSPHVR	1.398	24.776	1338
SPHGLR	0.000	89.878	1171	TSPHRM	0.741	30.175	1339
SPHALR	0.000	88.891	1172	GSPHVK	0.000	28.966	1340
SPHRVT	0.025	85.759	1173	SSPHGR	8.916	66.446	1341
SPHQRN	0.000	85.292	1174	VSPHGR	1.293	27.805	1342
SPHNNR	0.536	82.342	1175	SSPHRV	0.034	26.874	1343
SPHPGR	1.642	81.130	1176	TSPHFK	0.000	26.063	1344
SPHRPV	0.000	80.851	1177	QSPHRM	0.174	24.312	1345
SPHART	2.460	66.823	1178	HSPHQR	1.433	23.110	1346
SPHMRA	0.512	80.301	1179	TSPHNR	0.000	21.934	1347

SPHRTP	0.000	79.837	1180	ASPHVR	0.073	21.892	1348
SPHYRT	0.087	79.520	1181	ASPHGK	0.000	20.160	1349
SPHRLG	0.000	79.055	1182	GSPHPR	0.000	19.560	1350
SPHVRV	0.000	78.765	1183	SSPHLR	0.000	19.491	1351
SPHRLM	0.000	77.843	1184	LSPHKM	0.000	19.203	1352
SPHRNI	0.000	77.343	1185	NSPHRL	1.143	30.485	1353
SPHMKG	0.000	77.077	1186	ESPHQR	0.000	15.900	1354
SPHRPG	0.000	76.884	1187	MSPHGR	0.000	15.804	1355
SPHSSK	0.000	75.110	1188	GSPHLR	0.000	15.692	1356
SPHTAR	0.000	73.322	1189	QSPHRV	0.000	13.770	1357
SPHKRG	0.000	73.134	974	TSPHRQ	0.000	13.190	1358
SPHSKL	0.000	72.898	960	VSPHSR	0.068	12.727	1359
SPHSRS	1.440	77.310	1190	TSPHKN	0.000	12.060	1360
SPHVKL	0.000	72.753	1191	PSPHDR	0.000	11.806	1361
SPHMSR	0.351	71.746	1192	SSPHGF	0.000	11.243	1362
SPHKSH	0.000	71.658	1193	FSPHKS	0.000	11.234	1363
SPHTMR	4.281	68.773	1194	LSPHVR	0.755	11.202	1364
SPHRPI	0.000	68.674	1195	HSPHRV	0.691	9.571	1365
SPHVRA	1.964	59.916	1196	SSPHRP	0.172	9.074	1366
SPHLNR	0.051	67.780	1197	MSPHTR	0.068	8.910	1367
SPHKLV	0.000	66.794	1198	TSPHFR	0.589	8.376	1368
SPHLRP	4.221	65.572	1199	RSPHPT	0.047	8.050	1369
SPHSKA	6.026	65.262	941	LSPHHR	3.754	8.623	1370
SPHRVL	0.000	64.653	1200	NSPHRP	0.966	6.784	1371
SPHGPK	0.000	62.942	1201	RSPHSQ	0.692	5.824	1372
SPHSLR	2.699	76.479	952	LSPHRQ	0.000	5.414	1373
SPHRVM	2.087	62.464	1202	PSPHRG	0.701	4.504	1374
SPHRTG	0.000	61.994	1203	RSPHGM	0.157	2.660	1375
SPHRAF	0.000	61.964	1204	ASPHLR	0.000	2.430	1376
SPHRSM	0.000	61.313	1205	SESPHR	0.000	148.607	1377
SPHSRA	0.975	78.830	1572	RQSPHT	0.000	122.326	1378
SPHRSV	0.000	60.055	1206	SASPHR	0.000	100.626	1379
SPHQQR	0.000	57.873	1207	SRSPHS	1.269	56.272	1380
SPHIRV	1.161	57.377	1208	RVSPHA	0.036	76.942	1381
SPHKSW	0.000	57.323	4743	QRSPHA	0.000	73.811	1382
SPHLSR	6.426	62.165	1209	TMSPHR	2.742	68.255	1383
SPHSRF	0.080	56.902	1210	RQSPHG	0.000	68.372	1384
SPHRSS	0.000	56.721	1211	RSSPHN	0.000	61.247	1385
SPHAIR	3.000	75.219	1212	ASSPHR	0.000	60.163	1386
SPHNSR	1.734	90.965	1213	SLSPHR	2.408	57.052	1387
SPHIRP	0.000	55.191	1214	NVSPHR	0.080	55.912	1388
SPHSRV	0.000	53.670	1215	RVSPHS	4.440	118.525	1389
SPHRPH	0.000	53.566	1216	RTSPHT	0.000	52.798	1390
SPHRPS	0.000	53.415	1217	SVSPHR	0.067	51.102	1391
SPHKPW	1.159	52.878	1218	EASPHK	0.000	50.325	1392
SPHHKL	0.393	52.634	1219	HLSPHK	0.000	50.272	1393
SPHGTR	0.000	48.764	1220	DRSPHN	0.000	47.073	1394
SPHGHR	0.000	48.157	1221	PSSPHR	0.000	45.962	1395
SPHRAS	0.000	47.229	1222	ANSPHR	0.344	41.276	1396
SPHKNL	0.000	47.048	1223	ARSPHS	0.000	40.314	1397
SPHRVP	2.476	48.744	1224	RTSPHA	0.000	39.449	1398
SPHVRL	2.252	61.058	1225	RVSPHT	0.022	36.241	1399
SPHGAK	1.354	32.733	1226	RASPHS	0.000	35.363	1400

SPHRST	0.000	46.140	1227	ISSPHR	0.000	31.020	1401
SPHSRT	0.000	45.764	1228	QRSPHN	0.000	28.752	1402
SPHGSW	0.000	45.562	1229	TRSPHS	0.000	28.085	1403
SPHRLH	0.000	43.554	1230	SSSPHR	0.000	27.777	1404
SPHKRS	0.000	42.553	1231	HASPHR	0.000	26.519	1405
SPHLRI	0.087	42.218	1232	HLSPHR	0.000	25.994	1406
SPHYKM	0.000	40.425	1233	WGSPHS	0.000	25.970	1407
SPHMRV	2.329	52.942	1234	SGSPHK	0.000	24.675	1408
SPHHSY	0.000	40.124	1235	LSSPHR	0.446	22.339	1409
SPHKYR	7.793	45.723	1236	GGSPHR	0.000	21.550	1410
SPHKFK	0.870	39.747	1237	RTSPHW	0.000	20.442	1411
SPHAKA	0.000	38.774	4736	WQSPHN	0.000	20.418	1412
SPHLKT	0.669	38.234	1238	QKSPHA	0.000	19.795	1413
SPHVKN	0.000	37.280	1239	SHSPHR	1.565	18.095	1414
SPHLRA	1.670	43.509	1240	HMSPHR	0.000	16.916	1415
SPHHRA	1.904	52.273	1241	RYSPHG	0.948	12.311	1416
SPHYGR	0.562	36.636	1242	RVSPHG	0.000	15.006	1417
SPHVGR	0.000	36.194	1243	LPSPHR	0.000	14.249	1418
SPHVRP	2.776	79.813	1244	RVSPHV	2.089	26.905	1419
SPHVRT	2.566	60.859	1245	SFSPHR	1.117	11.983	1420
SPHQRM	0.000	35.164	1246	RSSPHM	0.000	13.109	1421
SPHLGR	0.122	33.876	1247	LLSPHR	1.014	12.476	1422
SPHKRM	0.000	33.277	1248	RSSPHA	0.000	12.147	1423
SPHMVR	1.858	32.888	1249	AVSPHR	2.953	11.977	1424
SPHGPF	0.000	32.823	1250	RNSPHL	0.067	10.754	1425
SPHMRN	0.000	30.905	1251	RLSPHV	2.654	12.476	1426
SPHVRS	0.000	30.662	1252	RYSPHT	0.966	10.508	1427
SPHKRT	0.000	29.048	1253	SRSPHG	0.957	10.486	1428
SPHPGK	0.745	24.759	1254	GRSPHG	0.051	9.639	1429
SPHTGR	0.000	24.416	1255	MRSPHS	0.000	9.351	1430
SPHVQR	0.000	24.083	1256	LASPHR	6.722	9.308	1431
SPHNAR	1.422	30.886	1257	YRSPHS	0.000	8.938	1432
SPHWPK	0.000	23.325	1258	KESPHR	0.000	8.859	1433
SPHIAK	0.000	22.058	1259	RTSPHL	0.000	7.886	1434
SPHAKF	0.000	21.628	1260	SVSPHG	0.000	7.111	1435
SPHARA	0.122	21.512	1261	MGSPHR	0.000	4.342	1436
SPHMRF	0.000	20.998	1262	RSSPHL	0.019	4.314	1437
SPHTRF	2.752	20.911	1263	NSVSPH	4.415	13.974	1438
SPHSRI	0.102	18.860	1264	LFKSPH	0.000	10.432	1439

Table 38. NGS fold-change of AAV capsid variants that comprised a peptide insert present immediately subsequent to position 453, numbered according to SEQ ID NO: 982, in HEK293T cells expressing ALPL and wild-type HEK293T cells relative to virus input

Sequence	Fold-Change in WT cells	Fold-Change in ALPL expressing-cells	SEQ ID NO:	Sequence	Fold-Change in WT cells	Fold-Change in ALPL expressing-cells	SEQ ID NO:
GGSPHR	0.048	279.537	1410	RVSPHP	0.000	4.423	1553
STSPHR	0.044	172.109	1440	RLSPHH	2.121	4.259	1554
QQSPHR	2.682	144.486	1441	SVSPHA	0.000	4.240	1555
QSSPHR	0.129	144.306	1442	EWSPHR	0.000	4.184	1556

QASPHR	1.850	137.092	1443	RISPHL	7.535	4.118	1557
MSSPHR	0.522	135.774	1444	YRSPHQ	0.077	4.106	1558
RSSPHP	6.193	130.551	1445	TRSPHG	0.306	4.059	1559
SQSPHR	0.030	126.880	1446	RYSPHT	0.000	4.055	1427
DSSPHR	2.632	125.537	1447	RLSPHL	0.000	3.985	1560
NKSPHK	0.032	121.569	1448	GASPHS	0.000	3.893	1561
NGSPHR	1.883	117.833	1449	LRSPHY	3.466	3.671	1562
NSSPHR	0.000	111.571	1450	ITSPHL	0.000	3.600	1563
YGSPHR	0.048	111.112	1451	GHSPHG	0.000	3.456	1564
SVSPHR	0.058	106.268	1391	SKSPHS	0.774	3.374	1565
PGSPHR	0.361	97.520	1452	KLSPHS	0.000	3.257	1566
RGSPHK	0.048	93.901	1453	RTSPHT	2.294	2.771	1390
NRSPHK	0.474	91.362	1454	RTSPHW	0.000	2.350	1411
ANSPHR	1.020	88.759	1396	SPHIPR	0.273	11.638	1567
HTSPHR	0.000	85.500	1455	SPHHGR	2.041	11.403	1102
PTSPHR	0.000	85.232	1456	SPHSGP	0.000	9.764	1568
TGSPHR	0.671	84.098	1457	SPHRGS	0.215	6.700	1138
GSSPHR	0.043	83.804	1458	SPHRMN	0.000	4.748	1569
LGSPHR	0.271	82.569	1459	SPHLRL	0.096	3.217	1570
KGSPHK	0.000	76.182	1460	SPHLKG	5.079	2.929	1571
LNSPHK	0.000	71.807	1461	SPHARL	0.000	2.713	1140
RSSPHY	1.392	70.191	1462	SPHSRA	0.211	2.644	1572
LASPHR	1.355	66.463	1431	SPHLSR	1.542	2.209	1209
HLSPHR	0.000	66.406	1406	GSPHKR	0.000	28.667	1286
RESPHR	2.110	60.907	1463	SSPHRH	0.000	25.739	1573
HSSPHR	0.068	58.533	1464	SSPHKV	0.670	25.214	1296
MTSPHR	0.000	46.356	1465	TSPHRQ	0.077	25.131	1358
SWSPHR	0.505	42.709	1466	LSPHKR	0.000	24.201	1574
QTSPHR	0.000	42.191	1467	TSPHRH	0.018	22.819	1575
SLSPHR	0.185	41.511	1387	GSPHYK	0.000	22.344	1576
THSPHR	1.787	40.793	1468	GSPHRT	1.053	20.315	1577
VASPHR	0.602	35.919	1469	TSPHNR	0.000	20.145	1347
HGSPHR	2.448	33.135	1470	TSPHTK	0.731	19.806	1578
RMSPHG	1.505	32.455	1471	GSPHSR	1.341	16.029	1297
ALSPHR	0.906	31.949	1472	SSPHAR	2.107	15.444	1579
RMSPHP	0.000	31.814	1473	SSPHSR	1.136	15.047	1291
VHSPHR	0.014	31.169	1474	LSPHRG	0.000	13.432	1580
RQSPHK	0.000	29.471	1475	GSPHAR	1.437	13.365	1581
RTSPHP	0.710	28.082	1476	SSPHMK	3.948	12.859	1582
YNSPHR	0.513	27.737	1477	TSPHKP	0.000	12.808	1583
WGSPHS	2.872	27.495	1407	ASPHRI	1.584	12.498	1584
SASPHR	0.000	27.467	1379	WSPHVK	0.847	11.816	1585
QVSPHR	0.000	27.276	1478	LSPHGR	0.053	11.726	1586
YRSPHK	0.000	27.247	1479	MSPHSR	3.078	11.638	1311
RASPHP	0.925	27.047	1480	TSPHYR	0.802	11.609	1587
PYSPHR	3.937	26.760	1481	RSPHSY	1.766	10.831	1588
LSSPHR	2.207	26.579	1409	HSPHRG	0.000	10.546	1589
WLSPHR	1.117	25.916	1482	GSPHSK	1.285	10.531	1590
LSSPHK	0.000	25.726	1483	RSPHKF	0.000	10.513	1591
VSSPHR	0.000	25.352	1484	GSPHRL	0.000	10.498	1305
RLSPHY	0.044	25.238	1485	MSPHHK	1.935	10.186	1592
RMSPHS	0.910	24.481	1486	TSPHAR	1.210	9.810	1593
RSSPHW	1.111	23.642	1487	HSPHRV	1.963	9.695	1365

RHSPHK	0.000	22.180	1488	ASPHSR	0.076	9.335	1594
IHSPHK	0.000	21.355	1489	MSPHMR	2.394	8.933	1595
NASPHR	3.725	21.188	1490	SSPHYH	0.558	8.892	1596
DVSPHR	0.080	20.496	1491	TSPHQR	0.000	7.721	1597
RASPHY	0.745	18.949	1492	ASPHGK	0.000	7.647	1349
RNSPHG	0.080	18.347	1493	ISPHAK	0.183	7.468	1598
HVSPHK	0.000	17.926	1494	ISPHQR	1.044	7.028	1599
ARSPHY	0.000	17.725	1495	QSPHVR	0.935	6.748	1600
RLSPHT	1.731	16.337	1496	LSPHAR	0.015	6.710	1601
RSSPHA	1.375	16.279	1423	LSPHRS	0.000	6.669	1602
IQSPHR	0.000	14.591	1497	MSPHRH	0.000	5.998	1603
HDSPHK	0.033	14.495	2	SSPHVR	1.484	5.796	1338
IRSPHK	0.000	14.021	1498	VSPHKQ	0.000	5.777	1604
ERSPHR	0.000	13.868	1499	LSPHTK	0.023	5.693	1605
RFSPHS	0.000	13.605	1500	VSPHRS	0.000	5.286	1320
LGSPHS	0.053	13.573	1501	HSPHSR	0.410	4.833	1606
RLSPHG	1.209	13.411	1502	NSPHGK	2.594	4.762	1607
RTSPHS	0.878	13.036	1503	TSPHVR	3.010	4.201	1608
TTSPHS	0.000	12.918	1504	GSPHRN	2.271	4.169	1321
SRSPHN	0.581	12.917	1505	LSPHLG	0.000	2.795	1609
RGSPHL	0.368	12.626	1506	ESPHRP	2.682	2.574	1610
WTSPHS	0.000	12.085	1507	RSPHDR	0.615	2.240	1611
PFSPHG	0.000	11.531	1508	RSPHLN	0.197	2.178	1612
HRSPHP	0.000	11.362	1509	RPSSPH	0.055	23.412	1613
VYSPHS	0.000	11.341	1510	LHGSPH	0.000	18.999	1614
RMSPHQ	2.454	11.249	1511	RLGSPH	0.000	16.897	1615
PRSPHG	0.151	11.164	1512	HYSSPH	2.011	16.859	1616
LRSPHS	0.000	10.939	1513	RFGSPH	0.000	14.025	1617
LASPHK	0.000	10.545	1514	RSASPH	0.000	13.429	1618
WASPHR	1.393	10.413	1515	VRYSPH	0.034	12.436	1619
RGSPHQ	0.000	10.357	1516	KTASPH	0.000	12.319	1620
WRSPHG	0.982	10.174	1517	VGSSPH	0.000	12.051	1621
VRSPHS	1.505	10.172	1518	HRTSPH	0.000	11.917	1622
RTSPHL	0.958	10.156	1434	TPRSPH	1.573	10.796	1623
PRSPHM	0.000	9.905	1519	LRSSPH	1.565	10.735	1624
VLSPHR	1.541	9.864	1520	LARSPH	8.877	10.720	1625
MESPHR	0.000	9.475	1521	YRASPH	0.000	10.413	1626
RQSPHI	0.834	8.999	1522	RSGSPH	0.000	9.925	1627
GTSPHA	0.060	8.895	1523	WGTSPH	6.236	9.142	1628
SVSPHQ	0.000	8.718	1524	RGSSPH	0.000	9.044	1629
PLSPHA	0.000	8.206	1525	WHSSPH	0.000	8.956	1630
PRSPHA	0.064	7.736	1526	SRGSPH	3.334	8.419	1631
PRSPHT	5.031	7.615	1527	IQGSPH	0.055	8.181	1632
STSPHS	0.016	7.532	1528	RPGSPH	1.884	7.848	1633
EKSPHR	0.000	7.441	1529	APASPH	0.000	7.793	1634
SRSPHA	0.041	7.429	1530	RQGSPH	0.280	7.572	1635
TLSPHS	0.860	7.418	1531	KTMSPH	0.020	7.505	1636
RSSPHS	0.237	7.259	1532	HWSSPH	0.000	6.985	1637
ARSPHG	0.623	6.927	1533	RYSSPH	0.000	6.314	1638
LGSPHQ	0.000	6.920	1534	RTGSPH	0.000	6.294	1639
MSSPHA	0.000	6.836	1535	RNNSPH	0.000	5.277	1640
RTSPHV	0.973	6.794	1536	APRSPH	0.192	5.258	1641
QRSPHA	0.782	6.738	1382	LRASPH	0.000	5.011	1642

RQSPHG	0.000	6.475	1384	PPGSPH	0.000	4.823	1643
RLSPHA	0.000	6.421	1537	ARGSPH	4.447	4.744	1644
AKSPHW	5.688	6.314	1538	VGGSPH	0.000	4.540	1645
QRSPHS	0.871	6.271	1539	TGRSPH	1.026	4.522	1646
HISPHR	0.000	6.150	1540	WPNSPH	1.068	4.475	1647
RGSPHS	0.000	6.115	1541	SWKSPH	0.285	4.130	1648
RNSPHH	0.376	6.072	1542	RSLSPH	0.055	4.087	1649
ALSPHS	0.000	5.698	1543	MKHSPH	0.192	4.049	1650
QPSPHR	0.000	5.682	1544	RSYSPH	0.418	3.961	1651
TLSPHV	0.000	5.417	1545	SYGSPH	1.053	3.873	1652
VPSPHR	0.000	5.374	1546	RTFSPH	0.365	3.301	1653
RASPHN	0.000	5.345	1547	VTGSPH	0.000	3.046	1654
IESPHR	0.077	5.073	1548	RTHSPH	0.072	2.809	1655
IRSPHP	2.574	5.058	1549	AQRSPH	1.588	2.773	1656
LYSPHR	2.042	5.024	1550	GGRSPH	0.464	2.710	1657
VRSPHY	1.787	4.606	1551	LWRSPH	0.000	2.503	1658
RMSPHV	2.570	4.603	1552				

Example 5. *In vivo* screen of TRACER AAV libraries in Mice and NHPs

[0449] A subset of the library of AAV capsid variants generated in Example 4 and other AAV capsid variants generated with an insertion of 6 amino acid sequences immediately subsequent to positions 453 and 455, relative to a reference sequence numbered according to SEQ ID NO: 138, 981, or 982, was screened *in vivo* in three strains of mice (BALB/c, C57BL/6, and CD1 outbred mice) and non-human primates (cynomolgus macaques (*Macaca fascicularis*)). The animals were injected intravenously with the library of the sub-selected variants. After a period *in vivo*, RNA was extracted from the brain of the NHPs and the brains and livers of mice. Following RNA recovery and RT-PCR amplification, a systematic NGS enrichment analysis was performed, and the peptides comprised within the variants were identified and the average fold-change (of NGS reads, counts per million) was calculated for each variant relative to virus input.

[0450] **Table 49** provides the fold-change data for the capsid variants that comprised a peptide insert present immediately subsequent to position 453 (e.g., present at positions 454-459), numbered according to SEQ ID NO: 982, that comprised an SPH motif within the insert sequence, and demonstrated a fold-change relative to input that was greater than the fold-change relative to input observed with wild-type AAV9 in the NHP brain. **Table 50** provides the fold-change data for the capsid variants that comprised a peptide insert present immediately subsequent to position 455 (e.g., present at positions 456-461), numbered according to SEQ ID NO: 981, that comprised an SPH motif within the insert sequence and demonstrated a fold-change relative to input that was greater than the fold-change relative to input observed with wild-type AAV9 in the NHP brain. Several variants in both **Table 49** and **Table 50** demonstrated cross-species compatibility as evidenced by increased fold-change values relative to input as compared to fold-change value relative to input for wild-type AAV9 in both mice and NHPs.

Table 49. NGS fold-change (FC) of AAV capsid variants that comprised a peptide insert present immediately subsequent to position 453, numbered according to SEQ ID NO: 982, in the brains and livers of mice and the brain of NHPs relative to virus input

Sequence	SEQ ID NO	FC in BALB/c Mice Brain	FC in C57/BL6 Mice Brain	FC in CD1 Mice Brain	FC in BALB/c Mice Liver	FC in C57/BL6 Mice Liver	FC in CD1 Mice Liver	FC in NHP Brain (1)	FC in NHP Brain (2)
HDSPHK	2	60.13	53.75	58.89	0.19	0.22	0.12	28.54	13.45
PGSPHR	1452	31.35	23.63	26.09	0.49	0.50	0.51	16.63	6.37
HSSPHR	1464	22.96	9.90	18.13	0.77	0.72	0.85	10.32	3.75
NGSPHR	1449	33.23	29.93	37.04	0.33	0.65	0.47	6.32	4.36
QSSPHR	1442	19.74	10.71	13.80	0.89	0.75	0.81	5.01	0.23
SQSPHR	1446	6.73	7.98	14.72	0.54	0.83	1.01	4.94	1.34
DSSPHR	1447	10.86	17.19	14.76	0.60	0.60	0.48	4.79	0.64
STSPHR	1440	26.90	11.78	15.78	1.04	0.78	0.81	4.58	2.62
MSSPHR	1444	9.78	4.18	6.57	1.14	1.36	1.20	3.83	1.33
GSPHAR	1581	3.00	1.25	2.41	1.47	1.49	1.38	3.76	0.39
LSSPHR	1409	9.59	6.99	5.72	0.84	0.86	0.89	3.04	0.00
GGSPHR	1410	18.24	14.38	14.17	0.76	0.83	0.69	2.81	1.06
ALSPHR	1472	2.48	3.43	3.68	0.70	0.61	1.02	2.81	0.64
GSSPHR	1458	30.33	11.47	18.64	0.91	0.90	0.87	2.78	1.98
ANSPHR	1396	7.21	2.08	6.28	0.77	0.80	0.81	2.71	0.56
YGSPHR	1451	22.54	15.39	19.20	0.57	0.65	0.56	2.63	1.18
HLSPHR	1406	3.73	5.30	4.61	0.83	0.84	0.80	2.46	0.42
GSPHSR	1297	0.39	0.80	1.24	0.54	0.92	1.06	1.96	0.84
IQSPHR	1497	0.55	1.46	2.28	0.31	0.75	1.13	1.90	1.11
QQSPHR	1441	3.18	4.05	8.14	0.32	0.80	1.02	1.88	3.14
QASPHR	1443	15.93	6.42	9.47	1.13	1.05	1.18	1.78	2.40
SPHKYQ	1659	0.10	0.30	0.33	0.26	0.46	0.35	1.75	2.04
NVKSPH	1660	0.54	0.79	0.46	0.61	0.93	0.92	1.68	0.00
GSPHRT	1577	0.68	0.89	1.22	0.89	0.76	0.84	1.37	2.41
KTINGSG QNQQT	6405	1.90	1.53	1.02	1.00	0.63	0.59	1.23	0.44

Table 50. NGS fold-change (FC) of AAV capsid variants that comprised a peptide insert present immediately subsequent to position 455, numbered according to SEQ ID NO: 981, in the brains and livers of mice and the brain of NHPs relative to virus input

Sequence	SEQ ID NO	FC in BALB/c Mice Brain	FC in C57/BL6 Mice Brain	FC in CD1 Mice Brain	FC in BALB/c Mice Liver	FC in C57/BL6 Mice Liver	FC in CD1 Mice Liver	FC in NHP Brain (1)	FC in NHP Brain (2)
SPHAKS	1166	85.20	54.77	54.16	0.40	0.42	0.39	37.58	14.19
SPHARV	1108	6.40	7.50	16.22	0.48	0.63	0.63	32.03	13.19
SPHAKA	4736	52.68	42.99	48.51	0.53	0.37	0.44	27.04	13.93
SPHKLV	1198	3.29	1.98	47.68	0.27	0.12	0.17	26.69	7.11
SPHSKA	941	88.46	43.38	54.81	0.47	0.31	0.42	25.40	14.20
SPHARQ	1145	29.25	28.24	29.28	0.84	0.83	0.73	24.67	6.61
SPHARM	947	2.35	4.63	21.40	0.38	0.48	0.74	17.18	6.42
SPHKSL	4740	1.08	2.67	32.72	0.17	0.19	0.28	16.61	4.96
SPHRTV	1169	3.60	2.64	11.02	0.31	0.36	0.41	15.38	2.95
SPHRTL	1662	0.94	1.58	19.17	0.38	0.23	0.40	11.94	5.48
SPHARG	1110	34.34	20.60	27.54	1.31	0.83	1.03	11.28	2.47
SPHKNL	1223	1.54	1.39	24.32	0.12	0.14	0.27	10.81	4.91
SPHANR	1129	26.27	15.98	14.68	1.06	0.77	0.91	10.12	3.97
SPHKSH	1193	85.87	45.31	45.08	0.43	0.30	0.34	9.49	5.15
SPHRVV	1663	2.29	0.81	2.60	0.37	0.45	0.47	9.28	2.80

SPHRYT	1131	24.45	6.71	12.53	0.62	0.43	0.52	8.72	2.78
SPHSRP	1128	9.46	15.64	7.20	0.81	0.92	0.70	8.20	4.95
SPHVRA	1196	11.60	9.05	5.64	1.45	1.27	1.09	7.66	1.82
SPHAMR	1104	11.06	5.62	8.25	0.92	0.99	1.14	7.52	2.06
SPHRAV	1163	2.23	1.03	3.41	0.28	0.27	0.25	6.35	5.35
SPHRSM	1205	10.73	8.46	40.16	0.54	0.36	0.58	6.02	7.09
SPHGKF	1143	2.43	7.22	9.49	0.47	0.59	0.86	5.38	1.99
SPHRMV	1139	1.39	0.37	4.66	0.33	0.44	0.46	5.00	1.78
SPHTYR	1147	2.94	7.80	2.88	0.61	1.29	0.73	4.91	0.38
SPHGRG	1123	14.77	10.64	10.07	1.57	1.53	1.29	4.84	1.68
SPHRAM	1664	2.76	2.03	16.32	0.39	0.50	0.72	4.67	1.51
SPHRVT	1173	0.47	0.34	1.00	0.32	0.43	0.40	4.39	2.72
SPHRGI	1107	0.16	1.10	9.19	0.21	0.24	0.59	4.28	2.16
SPHAQR	1105	24.84	4.75	13.27	1.25	0.60	1.19	4.27	6.18
SPHSIR	1153	5.75	2.06	3.39	1.36	0.83	1.23	4.21	1.91
SPHGRV	1152	2.64	4.68	6.30	0.52	0.74	0.78	4.20	4.47
SPHRGS	1138	1.26	6.85	12.42	0.42	0.70	0.75	4.15	2.25
SPHRGP	1100	2.49	3.58	7.53	0.39	0.59	0.69	3.94	0.68
SPHRVP	1224	1.85	4.44	3.02	0.39	0.49	0.48	3.75	1.62
SPHGRL	1127	0.44	2.96	8.92	0.30	0.38	0.74	3.73	3.73
SPHMRA	1179	5.48	3.93	2.08	1.35	1.18	0.68	3.72	1.15
SPHRAF	1204	4.80	8.08	8.95	0.63	0.79	0.60	3.26	2.60
SPHRGM	1112	0.39	0.70	6.45	0.31	0.39	0.49	3.23	0.58
SPHGPR	1159	12.11	3.44	5.12	1.43	1.11	1.14	3.19	1.44
SPHMRV	1234	3.02	4.47	1.21	0.75	0.77	0.34	3.11	0.33
SPHKFK	1237	5.91	20.31	9.76	0.40	0.29	0.20	3.01	2.62
SPHRPI	1195	0.18	0.92	2.79	0.37	0.57	0.80	2.96	0.27
SPHGRS	1103	20.94	14.48	8.95	0.97	1.16	0.80	2.80	1.97
SPHRPM	1156	3.52	0.86	3.43	0.52	0.55	0.65	2.73	0.25
SPHRGL	1111	0.68	1.00	5.33	0.20	0.29	0.70	2.40	3.88
SPHRGG	1101	2.47	2.76	7.16	0.45	0.45	0.61	2.12	0.74
SPHRAT	1106	4.20	4.08	6.92	0.43	0.65	0.47	2.01	4.01
ISPHGR	1661	0.26	0.04	0.25	0.29	0.50	0.56	1.58	1.54
GSPHKR	1286	4.87	5.20	5.05	0.35	0.29	0.40	1.55	0.00
NSPHKW	1290	3.80	4.54	8.14	0.22	0.45	0.56	1.30	0.82
KTINGSG QNQQT	6405	1.90	1.53	1.02	1.00	0.63	0.59	1.23	0.44

[0451] A library of AAV capsid variants were generated with an insertion of 7 amino acid sequences immediately subsequent to position 453, relative to a reference sequence numbered according to SEQ ID NO: 138, 981, or 982, was screened *in vivo* in three strains of mice (BALB/c, C57BL/6, and CD1 outbred mice) and non-human primates (cynomolgus macaques (*Macaca fascicularis*)). The animals were injected intravenously with the library of the sub-selected variants. After a period *in vivo*, RNA was extracted from the brain of the NHPs and the brains and livers of mice. Following RNA recovery and RT-PCR amplification, a systematic NGS enrichment analysis was performed, and the peptides comprised within the variants were identified and the average fold-change (of NGS reads, counts per million) was calculated for each variant relative to virus input.

[0452] **Table 51** provides the fold-change data relative to input or wild-type AAV9 for the capsid variants that comprised a peptide insert of 7 amino acids present immediately subsequent to position 453 (e.g., present at positions 454-460), numbered according to SEQ ID NO: 982, that comprised an

SPH motif within the insert sequence in the brains of mice and NHPs. Several variants in **Table 51** demonstrated cross-species compatibility as evidenced by increased fold-change values relative to wild-type AAV9 in both mice and NHPs.

Table 51. NGS fold-change (FC) of AAV capsid variants that comprised a peptide insert present immediately subsequent to position 453, numbered according to SEQ ID NO: 982, in the brains of mice and the brain of NHPs relative to virus input or average fold change in NHPs or mice relative to AAV9

Sequence	SEQ ID NO	FC relative to input in BALB/c Mice Brain	FC relative to input in C57/BL6 Mice Brain	FC relative to input in CD1 Mice Brain	FC relative to input in NHP Brain (1)	FC relative to input in NHP Brain (2)	Average FC in NHPs relative AAV9	Average FC in NHPs relative to AAV9
SASPHAR	1665	62.70	62.59	39.17	43.43	18.37	36.79	37.04
SSSPHSR	1666	19.00	37.15	21.52	42.79	14.17	33.91	17.49
LGSPHSK	1667	67.77	40.97	21.33	36.94	17.53	32.42	29.30
IGSPHAR	1668	74.86	41.80	40.75	24.97	8.27	19.78	35.45
SLSPHAK	1669	58.26	38.95	63.96	22.98	10.06	19.66	36.30
QISPHKI	1670	3.15	25.69	8.43	8.18	17.56	15.32	8.39
LSSPHVK	1671	9.17	6.30	18.09	12.02	4.91	10.08	7.56
IASPHSR	1672	1.35	6.89	1.44	7.86	5.82	8.14	2.18
SAASPHK	1673	13.03	8.13	7.07	2.38	2.63	2.98	6.36
GNSSPHH	1674	40.71	24.25	9.11	2.37	1.59	2.36	16.68
ALTRSPH	1675	0.19	0.10	0.63	0.85	2.80	2.17	0.21
DVSPHYR	1676	13.44	6.67	4.14	2.59	0.40	1.78	5.46
RTASPHR	1677	12.10	7.01	4.41	0.96	2.01	1.77	5.30
SSPHRLP	1678	0.33	0.95	0.55	1.60	0.54	1.27	0.41
SRASPHS	1679	0.92	0.33	0.76	1.08	0.98	1.22	0.45
AGMSPHR	1680	0.32	3.43	4.40	0.80	0.84	0.98	1.83
LDQSPHR	1681	6.92	16.86	7.23	1.55	0.00	0.92	6.99
WSSSPHR	1682	9.41	4.25	4.53	1.18	0.00	0.71	4.10
RLASPHK	1683	1.83	4.33	2.73	0.64	0.00	0.38	2.00
FRLSPHG	1684	0.13	0.00	0.15	0.20	0.35	0.33	0.06
GPRSPHF	1685	0.11	0.17	0.43	0.53	0.00	0.32	0.16
KTINGSG QNQQT	6405	1.90	1.53	1.02	1.23	0.44	1.00	1.00

[0453] A library of AAV capsid variants were generated with an insertion of 9 amino acid sequences immediately subsequent to position 453, relative to a reference sequence numbered according to SEQ ID NO: 138, 981, or 982, was screened *in vivo* in three strains of mice (BALB/c, C57BL/6, and CD1 outbred mice) and non-human primates (cynomolgus macaques (*Macaca fascicularis*)). The animals were injected intravenously with the library of the sub-selected variants. After a period *in vivo*, RNA was extracted from the brain of the NHPs and the brains and livers of mice. Following RNA recovery and RT-PCR amplification, a systematic NGS enrichment analysis was performed, and the peptides comprised within the variants were identified and the average fold-change (of NGS reads, counts per million) was calculated for each variant relative to virus input.

[0454] **Table 52** provides the fold-change data relative to input or wild-type AAV9 for the capsid variants that comprised a peptide insert of 9 amino acids present immediately subsequent to position

453 (e.g., present at positions 454-462), numbered according to SEQ ID NO: 982, that comprised an SPH motif within the insert sequence in the brains of mice and NHPs. Several variants in **Table 52** demonstrated cross-species compatibility as evidenced by increased fold-change values relative to wild-type AAV9 in both mice and NHPs.

Table 52. NGS fold-change (FC) of AAV capsid variants that comprised a peptide insert present immediately subsequent to position 453, numbered according to SEQ ID NO: 982, in the brains of mice and the brain of NHPs relative to virus input or average fold change in NHPs or mice relative to AAV9

Sequence	SEQ ID NO	FC relative to input in BALB/c Mice Brain	FC relative to input in C57/BL6 Mice Brain	FC relative to input in CD1 Mice Brain	FC relative to input in NHP Brain (1)	FC relative to input in NHP Brain (2)	Average FC in NHPs relative AAV9	Average FC in NHPs relative to AAV9
PMSPHSRSV	1686	7.91	14.99	13.67	32.97	10.43	25.83	8.24
EQTSPHRKL	1687	4.29	10.67	24.82	21.71	7.36	17.31	8.96
ASSPHSKAL	1688	6.82	11.76	6.19	19.12	7.22	15.68	5.58
QYSSPHKKV	1689	16.07	54.24	48.01	16.04	7.70	14.13	26.65
RPVSPHALG	1690	26.88	55.67	36.36	14.83	8.59	13.94	26.78
AGSPHAKVP	1691	14.47	9.44	7.52	11.28	11.47	13.54	7.08
LTVSPHKRG	1692	18.04	38.41	48.34	11.54	7.19	11.15	23.60
SGSTSPHYK	1693	55.00	33.90	17.93	7.16	3.02	6.06	24.06
PQNDSPHKY	1694	64.94	124.31	90.86	3.22	4.38	4.52	63.09
TNGSPHSRA	1695	0.89	12.34	19.86	0.69	6.86	4.49	7.45
GHNASPHSK	1696	133.91	72.21	57.98	4.42	2.99	4.41	59.48
VIGSPHSRS	1697	4.53	19.65	34.10	2.86	3.80	3.96	13.13
ATSPHHVRS	1698	11.10	8.47	6.65	4.58	1.98	3.91	5.90
APQDSPHRK	1699	32.02	64.29	35.46	3.30	3.13	3.83	29.68
MGNISPHAR	1700	34.00	60.88	38.60	4.94	1.48	3.82	30.06
FGSDSPHKL	1701	23.62	46.97	30.11	2.89	3.15	3.60	22.68
PASPHLRLS	1702	2.13	0.71	0.49	0.09	5.86	3.54	0.75
APTDSPHRK	1703	31.77	49.17	32.30	3.11	2.83	3.54	25.51
VTVGSPHSK	1704	40.30	20.29	23.31	2.14	3.78	3.53	18.90
PAVSSPHSK	1705	28.34	9.58	11.17	4.09	1.60	3.39	11.06
MAISPHSRS	1706	0.57	2.26	1.60	0.86	4.59	3.24	1.00
PSSPHGRVH	1707	8.13	11.80	2.56	3.84	1.17	2.98	5.07
SHPSSPHKR	1708	10.06	54.19	52.68	3.67	1.21	2.91	26.34
SPPSSPHKR	1709	27.15	101.68	57.77	3.66	0.92	2.72	42.03
APPDSPHRN	1710	10.47	5.60	1.78	0.74	3.55	2.55	4.02
RPASPHATF	1711	4.12	7.33	5.25	2.91	1.19	2.44	3.76
APPASPHRK	1712	5.64	9.04	14.84	2.38	1.51	2.32	6.65
TYNSPHKQH	1713	13.08	38.80	5.91	0.65	3.14	2.26	13.02
APPDSPHQK	1714	41.49	60.18	39.34	2.00	1.79	2.25	31.76
LGNSSPHAR	1715	74.93	83.04	53.53	0.65	3.13	2.25	47.64
AQPDSPHRK	1716	11.30	30.59	18.31	0.91	2.74	2.17	13.56
AFRASASPH	1717	1.16	1.15	0.32	1.89	0.82	1.61	0.59
APPESPHRK	1718	9.42	15.86	16.09	1.81	0.54	1.40	9.32
APPHSPHRK	1719	1.27	7.62	4.03	0.72	1.58	1.37	2.91
FVEGSPHKR	1720	12.64	26.26	14.59	0.64	1.35	1.18	12.05
APPDSPHRK	1721	18.17	35.97	19.94	1.49	0.43	1.14	16.69
KYKETSPHR	1722	4.92	3.69	0.23	1.23	0.00	0.73	1.99
APPDSPHRR	1723	17.36	13.52	13.66	0.25	0.39	0.38	10.03
WSSPHRPST	1724	0.00	0.11	0.29	0.47	0.00	0.28	0.09
SQYPSPHKS	1725	0.00	6.56	6.07	0.46	0.00	0.27	2.85

VSRGSPHLK	1726	0.26	2.48	1.15	0.31	0.00	0.18	0.88
GSYSPHKQW	1727	2.09	1.54	1.61	0.21	0.00	0.12	1.18
APPDSPHLK	1728	4.09	10.77	1.99	0.16	0.00	0.10	3.80
SKSSPHKMV	1729	2.06	0.52	1.11	0.13	0.00	0.08	0.83
TLDTSPHRR	1730	29.03	19.41	11.10	0.00	0.00	0.00	13.41
APPYSPHRK	1731	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KTINGSGQN QQT	6405	1.90	1.53	1.02	1.23	0.44	1.00	1.00

We claim:

1. An AAV capsid variant, comprising an amino acid sequence comprising SPH, wherein the SPH is present at positions 454-456, 455-457, 457-459, 458-460, or 459-461, numbered according to SEQ ID NO: 138, wherein the AAV capsid variant comprises an amino acid sequence at least 95% identical to the amino acid sequence of positions 203-736 of SEQ ID NO: 138.

2. The AAV capsid variant of claim 1, which comprises:

(i) the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1284-1439, 1567-1658;

(ii) an amino acid sequence comprising at least 4, 5, or 6 consecutive amino acids from any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1284-1439, or 1567-1658;

(iii) an amino acid sequence comprising at least one, two, or three but no more than four different amino acids, relative to any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1284-1439, 1567-1658; or

(iv) an amino acid sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1284-1439, 1567-1658.

3. The AAV capsid variant of claim 1 or 2, wherein the amino acid sequence is present:

(i) immediately subsequent to position 455, numbered according to SEQ ID NO: 138 or 981; or

(ii) immediately subsequent to position 453, numbered according to SEQ ID NO: 138 or 982.

7. The AAV capsid variant of any one of claims 1-6, wherein the amino acid sequence:

(i) is present at positions 456-461, numbered according to SEQ ID NO: 138 or 981; or

(ii) is present at positions 454-459, numbered according to SEQ ID NO: 138 or 982.

4. The AAV capsid variant of any one of claims 1-3, which comprises:

(i) the amino acid sequence of any one of SEQ ID NOs: 1284-1439, and wherein the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138 or 981; or

(ii) the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, 1567-1658, and wherein the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138 or 982.

5. The AAV capsid variant of any one of claims 1-4, which comprises:

(i) the amino acid sequence of any one of SEQ ID NOs: 1284-1439, and wherein the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 138 or 981; or

(ii) the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, 1567-1571, 1573-1658, and wherein the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 138 or 982.

6. An AAV capsid variant comprising an amino acid sequence having the following formula: $X_1X_2SPHX_3$, wherein X_3 does not comprise a basic amino acid, e.g., is not K or R; wherein the amino acid sequence is present in loop IV, wherein the AAV capsid variant comprises an amino acid sequence at least 95% identical to the amino acid sequence of positions 203-736 of SEQ ID NO: 138.

7. The AAV capsid variant of claim 6, wherein X_3 is not K or R.

8. The AAV capsid variant of claim 6 or 7, wherein X_3 is P, Y, G, S, W, T, A, N, L, Q, M, I, V, or H.

9. An AAV capsid variant comprising an amino acid sequence having the following formula: $X_1X_2SPHX_3$, wherein X_3 is P, Y, G, S, W, T, A, N, L, Q, M, I, V, or H; wherein the amino acid sequence is present in loop IV, wherein the AAV capsid variant comprises an amino acid sequence at least 95% identical to the amino acid sequence of positions 203-736 of SEQ ID NO: 138.

10. The AAV capsid variant of any one of claims 6-9, wherein loop IV comprises positions 449-460, numbered according to SEQ ID NO: 138).

11. The AAV capsid variant of any one of claims 6-10, wherein:

(i) X_1 is R, W, A, L, T, S, P, H, V, G, M, Q, I, Y, or K; and/or

(ii) X_2 is S, M, T, G, A, L, N, R, F, Y, Q, V, K, I, or H.

12. The AAV capsid variant of any one of claims 6-11, wherein X_1X_2 comprises RS, RM, RT, WG, RA, RL, RN, AR, RF, LG, TT, SR, RG, WT, PF, HR, VY, PR, LR, WR, VR, RQ, GT, SV, PL, ST, TL, MS, QR, AK, AL, IR, RV, RI, YR, TR, RY, GA, IT, GH, SK, KL.

13. The AAV capsid variant of any one of claims 6-12, wherein the amino acid sequence comprises:

(i) SPHP (SEQ ID NO: 3), SPHY (SEQ ID NO: 4), SPHG (SEQ ID NO: 5), SPHS (SEQ ID NO: 4700), SPHW (SEQ ID NO: 6), SPHT (SEQ ID NO: 7), SPHA (SEQ ID NO: 8), SPHN (SEQ ID NO: 9), SPHL (SEQ ID NO: 10), SPHQ (SEQ ID NO: 12), SPHM (SEQ ID NO: 13), SPHI (SEQ ID NO: 14), SPHV (SEQ ID NO: 15), or SPHH (SEQ ID NO: 16);

(ii) SSPH (SEQ ID NO: 17), MSPH (SEQ ID NO: 18), TSPH (SEQ ID NO: 19), GSPH (SEQ ID NO: 20), ASPH (SEQ ID NO: 21), LSPH (SEQ ID NO: 22), NSPH (SEQ ID NO: 23), RSPH (SEQ ID NO: 24), FSPH (SEQ ID NO: 25), YSPH (SEQ ID NO: 26), QSPH (SEQ ID NO: 27), VSPH (SEQ ID NO: 28),
KSPH (SEQ ID NO: 29), ISPH (SEQ ID NO: 30), or HSPH (SEQ ID NO: 31);

(iii) RSSPH (SEQ ID NO: 32), RMSPH (SEQ ID NO: 33), RTSPH (SEQ ID NO: 34), WGSPH (SEQ ID NO: 35), RASPH (SEQ ID NO: 37), RLSPH (SEQ ID NO: 38), RNSPH (SEQ ID NO: 40), ARSPH (SEQ ID NO: 41), RFSPH (SEQ ID NO: 42), LGSPH (SEQ ID NO: 43), TTSPH (SEQ ID NO: 44), SRSPH (SEQ ID NO: 45), RGSPH (SEQ ID NO: 46), WTSPH (SEQ ID NO: 47), PFSPH (SEQ ID NO: 48), HRSPH (SEQ ID NO: 49), VYSPH (SEQ ID NO: 50), PRSPH (SEQ ID NO: 53), LRSPH (SEQ ID NO: 54), WRSPH (SEQ ID NO: 55), VRSPH (SEQ ID NO: 56), RQSPH (SEQ ID NO: 57), GTSPH (SEQ ID NO: 58), SVSPH (SEQ ID NO: 59), PLSPH (SEQ ID NO: 60), STSPH (SEQ ID NO: 61), TLSPH (SEQ ID NO: 62), MSSPH (SEQ ID NO: 63), QRSPH (SEQ ID NO: 64), AKSPH (SEQ ID NO: 65), ALSPH (SEQ ID NO: 66), IRSPH (SEQ ID NO: 67), RVSPH (SEQ ID NO: 68), RISPH (SEQ ID NO: 69), YRSPH (SEQ ID NO: 70), TRSPH (SEQ ID NO: 71), RYSPH (SEQ ID NO: 72), GASPH (SEQ ID NO: 73), ITSPH (SEQ ID NO: 74), GHSPH (SEQ ID NO: 75), SKSPH (SEQ ID NO: 76), or KLSPH (SEQ ID NO: 77); and/or

(iv) SSPHP (SEQ ID NO: 78), SSPHY (SEQ ID NO: 79), MSPHG (SEQ ID NO: 80), MSPHP (SEQ ID NO: 81), TSPHP (SEQ ID NO: 82), GSPHS (SEQ ID NO: 83), ASPHP (SEQ ID NO: 84), LSPHY (SEQ ID NO: 85), MSPHS (SEQ ID NO: 86), SSPHW (SEQ ID NO: 87), ASPHY (SEQ ID NO: 88), NSPHG (SEQ ID NO: 89), RSPHY (SEQ ID NO: 90), LSPHT (SEQ ID NO: 91), SSPHA (SEQ ID NO: 92), FSPHS (SEQ ID NO: 93), LSPHG (SEQ ID NO: 94), TSPHS (SEQ ID NO: 95), RSPHN (SEQ ID NO: 96), GSPHL (SEQ ID NO: 97), FSPHG (SEQ ID NO: 98), RSPHP (SEQ ID NO: 99), YSPHS (SEQ ID NO: 100), MSPHQ (SEQ ID NO: 101), RSPHG (SEQ ID NO: 102), RSPHS (SEQ ID NO: 103), GSPHQ (SEQ ID NO: 104), TSPHL (SEQ ID NO: 105), RSPHM (SEQ ID NO: 106), QSPHI (SEQ ID NO: 107), TSPHA (SEQ ID NO: 108), VSPHQ (SEQ ID NO: 109), LSPHA (SEQ ID NO: 110), RSPHA (SEQ ID NO: 111), RSPHT (SEQ ID NO: 112), LSPHS (SEQ ID NO: 113), SSPHS (SEQ ID NO: 114), TSPHV (SEQ ID NO: 115), QSPHG (SEQ ID NO: 116), KSPHW (SEQ ID NO: 117), NSPHH (SEQ ID NO: 118), LSPHV (SEQ ID NO: 119), ASPHN (SEQ ID NO: 120), MSPHV (SEQ ID NO: 121), VSPHP (SEQ ID NO: 122), LSPHH (SEQ ID NO: 123), VSPHA (SEQ ID NO: 124), ISPHL (SEQ ID NO: 125), RSPHQ (SEQ ID NO: 126), YSPHT (SEQ ID NO: 127), LSPHL (SEQ ID NO: 128), ASPHS (SEQ ID NO: 129), HSPHG (SEQ ID NO: 130), KSPHS (SEQ ID NO: 131), TSPHT (SEQ ID NO: 132), or TSPHW (SEQ ID NO: 133).

14. The AAV capsid variant of any one of claims 1-13, which comprises the amino acid sequence of any one of SEQ ID NOs: 1382, 1384, 1390, 1407, 1411, 1423, 1427, 1434, 1445, 1462, 1471, 1473,

1476, 1480, 1485-1487, 1492, 1493, 1495, 1496, 1500-1513, 1516-1519, 1522-1528, 1530-1539, 1541-1543, 1545, 1547, 1549, 1551-1555, or 1557-1566.

15. An AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1100-1571, 1573-1658, or 1659-1735, wherein the amino acid sequence is present immediately subsequent to position 453 or 455, numbered according to SEQ ID NO: 138 or 981, wherein the AAV capsid variant comprises an amino acid sequence at least 95% identical to the amino acid sequence of positions 203-736 of SEQ ID NO: 138.

16. The AAV capsid variant of claim 15, which comprises:

(i) the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, 1379, 1382, 1384, 1387, 1390, 1391, 1396, 1406, 1407, 1409, 1410, 1411, 1423, 1427, 1431, 1434, 1440-1571, or 1573-1658;

(ii) the amino acid sequence comprises any one of SEQ ID NOs: 1379, 1387, 1391, 1396, 1406, 1409, 1410, 1431, 1440-1444, 1446-1461, 1463-1470, 1472, 1474, 1475, 1477-1479, 1481-1484, 1488-1491, 1494, 1497-1499, 1514, 1515, 1520, 1521, 1529, 1540, 1544, 1546, 1548, 1550, or 1556;

(iii) the amino acid sequence of any one of SEQ ID NOs: 1382, 1384, 1390, 1407, 1411, 1423, 1427, 1434, 1445, 1462, 1471, 1473, 1476, 1480, 1485-1487, 1492, 1493, 1495, 1496, 1500-1513, 1516-1519, 1522-1528, 1530-1539, 1541-1543, 1545, 1547, 1549, 1551-1555, or 1557-1566;

(iv) the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1567, 1568, 1569, 1570, or 1571;

(v) the amino acid sequence of any one of SEQ ID NOs: 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, or 1573-1612;

(vi) the amino acid sequence comprises any one of SEQ ID NOs: 1613-1658;

(vii) the amino acid sequence comprises any one of SEQ ID NOs: 1665-1685;

(viii) the amino acid sequence comprises any one of SEQ ID NOs: 1686-1731;

(ix) the amino acid sequence comprises any one of SEQ ID NOs: 1732-1735.

17. The AAV capsid variant of any one of claims 6-16, wherein the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138 or 982.

18. The AAV capsid variant of any one of claims 6-17, wherein the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 138 or 982.

19. The AAV capsid variant of any one of claims 6-14, 17, or 18, wherein X_1 is present at position 454, X_2 is present at position 455, S is present at position 456, P is present at position 457, H is

present at position 458, and X_3 is present at position 459, numbered according to SEQ ID NO: 138 or 982.

20. Th e AAV capsid variant of claim 15, which comprises:

(i) the amino acid sequence of any one of SEQ ID NOs: 1100-1439;

(ii) the amino acid sequence of any one of SEQ ID NOs: 1100-1283;

(iii) the amino acid sequence of any one of SEQ ID NOs: 1284-1376;

(iv) the amino acid sequence of any one of SEQ ID NOs: 1377-1437; or

(v) the amino acid sequence of SEQ ID NO: 1438 or 1439.

21. The AAV capsid variant of claim 15 or 20, wherein the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138 or 981.

22. The AAV capsid variant of any one of claims 15, 20, or 21, wherein the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 138 or 981.

23. An AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1102, 1138, 1140, 1209, 1286, 1291, 1296, 1297, 1305, 1311, 1320, 1321, 1338, 1347, 1349, 1358, 1365, 1379, 1382, 1384, 1387, 1390, 1391, 1396, 1406, 1407, 1409, 1410, 1411, 1423, 1427, 1431, 1434, 1440-1571, or 1573-1731, wherein the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138 or 982, wherein the AAV capsid variant comprises an amino acid sequence at least 95% identical to the amino acid sequence of positions 203-736 of SEQ ID NO: 138.

24. An AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1379, 1387, 1391, 1396, 1406, 1409, 1410, 1431, 1440-1444, 1446-1461, 1463-1470, 1472, 1474, 1475, 1477-1479, 1481-1484, 1488-1491, 1494, 1497-1499, 1514, 1515, 1520, 1521, 1529, 1540, 1544, 1546, 1548, 1550, or 1556, wherein the amino acid sequence is present immediately subsequent to position 453, numbered according to SEQ ID NO: 138 or 982, wherein the AAV capsid variant comprises an amino acid sequence at least 95% identical to the amino acid sequence of positions 203-736 of SEQ ID NO: 138.

25. The AAV capsid variant of claim 23 or 24, wherein the amino acid sequence is present at positions 454-459, numbered according to SEQ ID NO: 138 or 982.

26. An AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1100-1439, wherein the amino acid sequence is present immediately subsequent to position 455, numbered

according to SEQ ID NO: 138 or 981, wherein the AAV capsid variant comprises an amino acid sequence at least 95% identical to the amino acid sequence of positions 203-736 of SEQ ID NO: 138.

27. An AAV capsid variant comprising the amino acid sequence of any one of SEQ ID NOs: 1100-1283, wherein the amino acid sequence is present immediately subsequent to position 455, numbered according to SEQ ID NO: 138 or 981, wherein the AAV capsid variant comprises an amino acid sequence at least 95% identical to the amino acid sequence of positions 203-736 of SEQ ID NO: 138.

28. The AAV capsid variant of claim 26 or 27, wherein the amino acid sequence is present at positions 456-461, numbered according to SEQ ID NO: 138 or 981.

29. An AAV capsid variant comprising an amino acid sequence comprising:

(a) at least 4, 5, or 6 consecutive amino acids of any one of the sequences in Tables 1, 48, or 49;

(b) at least 4, 5, or 6 consecutive amino acids of any one of SEQ ID NOs: 1100-1571, 1573-1664, or 1732-1735;

(c) at least 4, 5, 6, or 7 consecutive amino acids of any one of SEQ ID NOs: 1665-1685; or

(c) at least 4, 5, 6, 7, 8 or 9 consecutive amino acids of any one of SEQ ID NOs: 1686-1731, optionally wherein the amino acid sequence is present:

(i) in hypervariable loop IV;

(ii) immediately subsequent to position 453 or 455, numbered according to SEQ ID NO: 138.

30. The AAV capsid variant of claim 29, wherein the amino acid sequence comprises:

(a) the amino acid sequence of any one of the sequences in Tables 1, 48, or 49;

(b) the amino acid sequence of any one of SEQ ID NOs: 1100-1571, 1573-1664, or 1732-1735;

(c) the amino acid sequence of any one of SEQ ID NOs: 1665-1685; or

(c) the amino acid sequence of SEQ ID NOs: 1686-1731.

31. The AAV capsid variant of any one of the preceding claims, which comprises one, two, or all of the following properties:

(i) is capable of binding to a glycosylphosphatidylinositol (GPI) anchored protein, e.g., alkaline phosphatase (ALPL);

(ii) demonstrates preferential transduction in a cell comprising a glycosylphosphatidylinositol (GPI) anchored protein, e.g., alkaline phosphatase (ALPL), relative to a cell that does not comprise a glycosylphosphatidylinositol (GPI) anchored protein, e.g., as measured by an assay, e.g., an assay of Example 4; and/or

(iii) is enriched at least 292, 250, 230, 220, 215, 210, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 105, 100, 90, 95, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, 5, 4, 3, or 2-fold in a cell comprising a glycosylphosphatidylinositol (GPI) anchored protein, e.g., alkaline phosphatase (ALPL), e.g., relative to levels present prior to transduction, e.g., as measured by an assay, e.g., an assay of Example 4.

32. The AAV capsid variant of claim 31, wherein the GPI anchored protein:

(i) is conserved in at least two to three species, e.g., at least three species (e.g., mice, NHPs (e.g., *Macaca fascicularis*), and/or humans);

(ii) is present on the surface of a cell in the blood brain barrier; and/or

(iii) is ALPL.

33. The AAV capsid variant of any one of the preceding claims, which further comprises:

(i) a modification, e.g., an insertion, substitution (e.g., conservative substitution), and/or deletion, in loop I, II, VI and/or VIII; and/or

(ii) a substitution at position K449, e.g., a K449R substitution, numbered according to SEQ ID NO: 138.

34. The AAV capsid variant of any one of claims 1-33, wherein the AAV capsid variant comprises an amino acid sequence at least 98% identical to the amino acid sequence of positions 203-736 of SEQ ID NO: 138.

35. The AAV capsid variant of any one of claims 1-34, wherein:

(ii) the AAV capsid variant comprises an amino acid sequence at least 95% or 98% identical to the amino acid sequence of positions 138-736 of SEQ ID NO: 138;

(iv) the AAV capsid variant further comprises an amino acid sequence comprising at least one, two or three, but no more than 30, 20 or 10 different amino acids relative to the amino acid sequence of SEQ ID NO: 138; and/or

(vi) the AAV capsid variant further comprises an amino acid sequence at least 95% or 98% identical to SEQ ID NO: 138.

36. The AAV capsid variant of any one of claims 1-8, 20-22, or 26-35, which comprises:

(i) the amino acid sequence of positions 203-455 of SEQ ID NO: 138 and the amino acid sequence of positions 456-736 of SEQ ID NO: 138;

(ii) the amino acid sequence of positions 138-455 of SEQ ID NO: 138 and the amino acid sequence of positions 456-736 of SEQ ID NO: 138; and/or

(iii) the amino acid sequence of positions 1-455 of SEQ ID NO: 138 and the amino acid sequence of positions 456-736 of SEQ ID NO: 138.

37. The AAV capsid variant of any one of claims 1-19, 23-25, or 29-35, which comprises:

(i) the amino acid sequence of positions 203-453 of SEQ ID NO: 138 and the amino acid sequence of positions 454-736 of SEQ ID NO: 138;

(ii) the amino acid sequence of positions 138-453 of SEQ ID NO: 138 and the amino acid sequence of positions 454-736 of SEQ ID NO: 138; and/or

(iii) the amino acid sequence of positions 1-453 of SEQ ID NO: 138 and the amino acid sequence of positions 454-736 of SEQ ID NO: 138.

38. A polynucleotide encoding the AAV capsid variant of any one of claims 1-37.

39. A peptide comprising the amino acid sequence of any one of SEQ ID NOs: 1100-1571 or 1573-1735.

40. A peptide comprising an amino acid sequence comprising:

(a) at least 4, 5, or 6 consecutive amino acids of any one of the sequences in Tables 1, 48, or 49;

(b) at least 4, 5, or 6 consecutive amino acids of any one of SEQ ID NOs: 1100-1571, 1573-1664, or 1732-1735;

(c) at least 4, 5, 6, or 7 consecutive amino acids of any one of SEQ ID NOs: 1665-1685; or

(c) at least 4, 5, 6, 7, 8 or 9 consecutive amino acids of any one of SEQ ID NOs: 1686-1731.

41. The peptide of claim 40, wherein the amino acid sequence comprises:

(a) the amino acid sequence of any one of the sequences in Tables 1, 48, or 49;

(b) the amino acid sequence of any one of SEQ ID NOs: 1100-1571, 1573-1664, or 1732-1735;

(c) the amino acid sequence of any one of SEQ ID NOs: 1665-1685; or

(c) the amino acid sequence of SEQ ID NOs: 1686-1731.

42. An AAV particle comprising the AAV capsid variant of any one of claims 1-37, an AAV capsid variant encoded by the polynucleotide of claim 38, or an AAV capsid variant comprising the peptide of any one of claims 39-41.

43. The AAV particle of claim 42, which comprises a nucleotide sequence encoding a payload, optionally wherein the encoded payload comprises a therapeutic protein or functional variant thereof;

an antibody or antibody fragment; an enzyme; a component of a gene editing system; an RNAi agent (e.g., a dsRNA, siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA); or a combination thereof.

44. The AAV particle of claim 43, wherein:

(i) the therapeutic protein or functional variant thereof, e.g., a recombinant protein, is associated with (e.g., aberrantly expressed in) a neurological or neurodegenerative disorder, a muscular or neuromuscular disorder, or a neuro-oncological disorder, optionally wherein the therapeutic protein or functional variant thereof is chosen from apolipoprotein E (APOE) (e.g., ApoE2, ApoE3 and/or ApoE4); human survival of motor neuron (SMN) 1 or SMN2; aromatic L-amino acid decarboxylase (AADC); aspartoacylase (ASPA); tripeptidyl peptidase I (CLN2); beta-galactosidase (GLB1); N-sulphoglucosamine sulphohydrolase (SGSH); N-acetyl-alpha-glucosaminidase (NAGLU); iduronate 2-sulfatase (IDS); intracellular cholesterol transporter (NPC1); gigaxonin (GAN); or a combination thereof;

(ii) the antibody or antibody binding fragment binds to

(a) a CNS related target, e.g. an antigen associated with a neurological or neurodegenerative disorder, e.g., β-amyloid, APOE, tau, SOD1, TDP-43, huntingtin (HTT), and/or synuclein;

(b) a muscular or neuromuscular related target, e.g., an antigen associated with a muscular or neuromuscular disorder; or

(c) a neuro-oncology related target, e.g., an antigen associated with a neuro-oncological disorder, e.g., HER2, or EGFR (e.g., EGFRvIII);

(iii) the enzyme comprises a meganuclease, a zinc finger nuclease, a TALEN, a recombinase, integrase, a base editor, a Cas9, or a fragment thereof;

(iv) the component of a gene editing system comprises one or more components of a CRISPR-Cas system, optionally wherein the one or more components of the CRISPR-Cas system comprises a Cas9, e.g., a Cas9 ortholog or a Cpf1, and a single guide RNA (sgRNA), wherein:

(a) the sgRNA is located upstream (5') of the cas9 enzyme; and/or

(b) the sgRNA is located downstream (3') of the cas9 enzyme; and/or

(v) the RNAi agent (e.g., a dsRNA, siRNA, shRNA, pre-miRNA, pri-miRNA, miRNA, stRNA, lncRNA, piRNA, or snoRNA), modulates, e.g., inhibits, expression of, a CNS related gene, mRNA, and/or protein, optionally wherein the CNS related gene is chosen from SOD1, MAPT, APOE, HTT, TDP-43, APP, BACE, SNCA, ATXN1, ATXN3, ATXN7, SCN1A-SCN5A, SCN8A-SCN11A, or a combination thereof.

45. The AAV particle of any one of claims 42-44, which comprises a viral genome comprising a promoter operably linked to the nucleic acid sequence encoding the payload, optionally wherein:

(i) the promoter is chosen from human elongation factor 1α-subunit (EF1α), cytomegalovirus (CMV) immediate-early enhancer and/or promoter, chicken β-actin (CBA) and its derivative CAG, β glucuronidase (GUSB), or ubiquitin C (UBC), neuron-specific enolase (NSE), platelet-derived growth factor (PDGF), platelet-derived growth factor B-chain (PDGF-β), intercellular adhesion molecule 2 (ICAM-2), synapsin (Syn), methyl-CpG binding protein 2 (MeCP2), Ca2+/calmodulin-dependent protein kinase II (CaMKII), metabotropic glutamate receptor 2 (mGluR2), neurofilament light (NFL) or heavy (NFH), β-globin minigene nβ2, preproenkephalin (PPE), enkephalin (Enk) and excitatory amino acid transporter 2 (EAAT2), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), 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 fragment, e.g., a truncation, or a functional variant thereof;

(ii) the promoter is an EF-1a promoter variant, e.g., a truncated EF-1a promoter; or

(iii) the promoter comprises the nucleotide sequence of any one of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998-1007 or any one of the sequences provided in Table 8, a nucleotide sequence comprising at least one, two, or three but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998-1007 or any one of the sequences provided in Table 8, or a nucleotide sequence with at least 80% (e.g., 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 987, 988, 990, 991, 995, 996, 998-1007 or any one of the sequences provided in Table 8.

46. The AAV particle of claim 45, wherein the viral genome further comprises:

(i) a polyA signal sequence;

(ii) an inverted terminal repeat (ITR) sequence, optionally wherein the ITR sequence is positioned 5' relative to the encoded payload and/or the ITR sequence is positioned 3' relative to the encoded payload;

(iii) an enhancer, a Kozak sequence, an intron region, and/or an exon region;

(iv) a nucleotide sequence encoding a miR binding site, e.g., a miR binding site that modulates, e.g., reduces, expression of the antibody molecule encoded by the viral genome in a cell or tissue where the corresponding miRNA is expressed, optionally wherein the encoded miR binding site modulates, e.g., reduces, expression of the encoded antibody molecule in a cell or tissue of the DRG, liver, heart, hematopoietic lineage, or a combination thereof; and/or

(v) a nucleotide sequence encoding a Rep protein, e.g., a non-structural protein, wherein the Rep protein comprises a Rep78 protein, a Rep68, Rep52 protein, and/or a Rep40 protein, optionally wherein the Rep78 protein, the Rep68 protein, the Rep52 protein, and/or the Rep40 protein are encoded by at least one Rep gene.

the viral genome further comprises a polyA signal sequence.

47. The AAV particle of any one of claims 45 or 46, wherein the viral genome comprises:

(i) at least 1-5 copies of the encoded miR binding site, e.g., at least 1, 2, 3, 4, or 5 copies;

(ii) at least 3 copies of an encoded miR binding sites, optionally wherein:

(a) all three copies comprise the same miR binding site, or at least one, two, three, or all of the copies comprise a different miR binding site; and/or

(b) the 3 copies of the encoded miR binding sites are continuous (e.g., not separated by a spacer), or are separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of GATAGTTA; or

(iii) at least 4 copies of an encoded miR binding site, optionally wherein

(a) all four copies comprise the same miR binding site, or at least one, two, three, or all of the copies comprise a different miR binding site; and/or

(b) the 4 copies of the encoded miR binding sites are continuous (e.g., not separated by a spacer), or are separated by a spacer, optionally wherein the spacer comprises the nucleotide sequence of GATAGTTA, or a nucleotide sequence having at least one, two, or three modifications, e.g., substitutions (e.g., conservative substitutions), but no more than four modifications, e.g., substitutions (e.g., conservative substitutions), relative to the nucleotide sequence of GATAGTTA.

48. The AAV particle of claim 46 or 47, wherein the encoded miR binding site comprises a miR122 binding site, a miR183 binding site, a miR-1 binding site, a miR-142-3p, or a combination thereof, optionally wherein:

(i) the encoded miR122 binding site comprises the nucleotide sequence of SEQ ID NO: 4673, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4673;

(ii) the encoded miR183 binding site comprises the nucleotide sequence of SEQ ID NO: 4676, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4676;

(iii) the encoded miR-1 binding site comprises the nucleotide sequence of SEQ ID NO: 4679, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4679; and/or

(iv) the encoded miR-142-3p binding site comprises the nucleotide sequence of SEQ ID NO: 4675, or a nucleotide sequence substantially identical (e.g., having at least 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% sequence identity) thereto; or a nucleotide sequence having at least one, two, three, four, five, six, or seven modifications, e.g., substitutions (e.g., conservative substitutions), but no more than ten modifications, e.g., substitutions (e.g., conservative substitutions), relative to SEQ ID NO: 4675.

49. The AAV particle of any one of claims 45-48, wherein the viral genome:

(i) is single stranded;

(ii) is self-complementary; and/or

(ii) further comprises a nucleic acid encoding the AAV capsid variant of any one of claims 1-35.

50. The AAV capsid variant, polynucleotide, peptide, or AAV particle of any one of the preceding claims which is isolated, e.g., recombinant.

51. A vector comprising a polynucleotide encoding the AAV capsid variant of any one of claims 1-37 or 50, the polynucleotide of claim 38 or 50, or a polynucleotide encoding the peptide of any one of claims 39-41 or 50.

52. A cell, e.g., a host cell, comprising the AAV capsid variant of any one of claims 1-37 or 50, the polynucleotide of claim 38 or 50, the peptide of any one of claims 39-41 or 50, the AAV particle of any one of claims 42-50, or the vector of claim 51, optionally wherein:

(i) the cell is a mammalian cell or an insect cell;

(ii) the cell is a cell of a brain region or a spinal cord region, optionally a cell of the brain stem, hippocampus, or thalamus; and/or

(iii) the cell is a neuron, a sensory neuron, a motor neuron, an astrocyte, a glial cell, oligodendrocyte, or a muscle cell (e.g., a cell of the heart, diaphragm, or quadriceps).

53. A method of making an AAV particle, comprising

(i) providing a host cell comprising a viral genome; and

(ii) incubating the host cell under conditions suitable to enclose the viral genome in the AAV capsid variant of any one of claims 1-37 or 50, or an AAV capsid variant encoded by the polynucleotide of claim 38 or 50;

thereby making the AAV particle.

54. A pharmaceutical composition comprising the AAV particle of any one of claims 38-46, an AAV particle comprising the AAV capsid variant of any one of claims 1-37 or 50, an AAV particle comprising the peptide of any one of claims 39-41 or 50, and a pharmaceutically acceptable excipient.

55. A method of delivering a payload to a cell or tissue (e.g., a CNS cell or a CNS tissue), comprising administering an effective amount of the pharmaceutical composition of claim 54, the AAV particle of any one of claims 42-50, an AAV particle comprising the capsid variant of any one of claims 1-37 or 50, or an AAV particle comprising the peptide of any one of claims 39-41 or 50.

56. The method of claim 55, wherein the cell is:

(i) a cell of a brain region or a spinal cord region, optionally a cell of the frontal cortex, sensory cortex, motor cortex, caudate, cerebellar cortex, cerebral cortex, brain stem, hippocampus, or thalamus;

(ii) a neuron, a sensory neuron, a motor neuron, an astrocyte, a glial cell, or an oligodendrocyte; and/or

(iii) within a subject, optionally wherein the subject has, has been diagnosed with having, or is at risk of having a neurological disorder, e.g., a neurodegenerative disorder, a neuro-oncological disorder, a muscular disorder, or a neuromuscular disorder.

57. A method of treating a subject having or diagnosed with having a neurological disorder, e.g., a neurodegenerative disorder, a neuro-oncological disorder, a muscular disorder, or a neuromuscular disorder, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 54, the AAV particle of any one of claims 42-50, an AAV particle comprising the capsid variant of any one of claims 1-37 or 50, or an AAV particle comprising the peptide of any one of claims 39-41 or 50.

58. The method of claim 56 or 57, wherein the neurological disorder, neurodegenerative disorder, muscular disorder, neuromuscular disorder, or neuro-oncological disorder is Huntington's Disease, Amyotrophic Lateral Sclerosis (ALS), Gaucher Disease, Dementia with Lewy Bodies, Parkinson's disease, Spinal Muscular Atrophy, Alzheimer's Disease, a leukodystrophy (e.g., Alexander disease, autosomal dominant leukodystrophy with autonomic diseases (ADLD), Canavan disease, cerebrotendinous xanthomatosis (CTX), metachromatic leukodystrophy (MLD), Pelizaeus-

Merzbacher disease, or Refsum disease), or a cancer (e.g., a HER2/neu positive cancer or a glioblastoma).

59. The method of claim 57 or 58, where treating comprises prevention of progression of the disease or disorder in the subject, optionally wherein the subject is a human.

60. The method of any one of claims 55-59, wherein the AAV particle is administered to the subject:

(i) intravenously, via intra-cisterna magna injection (ICM), intracerebrally, intrathecally, intracerebroventricularly, via intraparenchymal administration, or intramuscularly;

(ii) via focused ultrasound (FUS), e.g., coupled with the intravenous administration of microbubbles (FUS-MB), or MRI-guided FUS coupled with intravenous administration; or

(iii) intravenously.

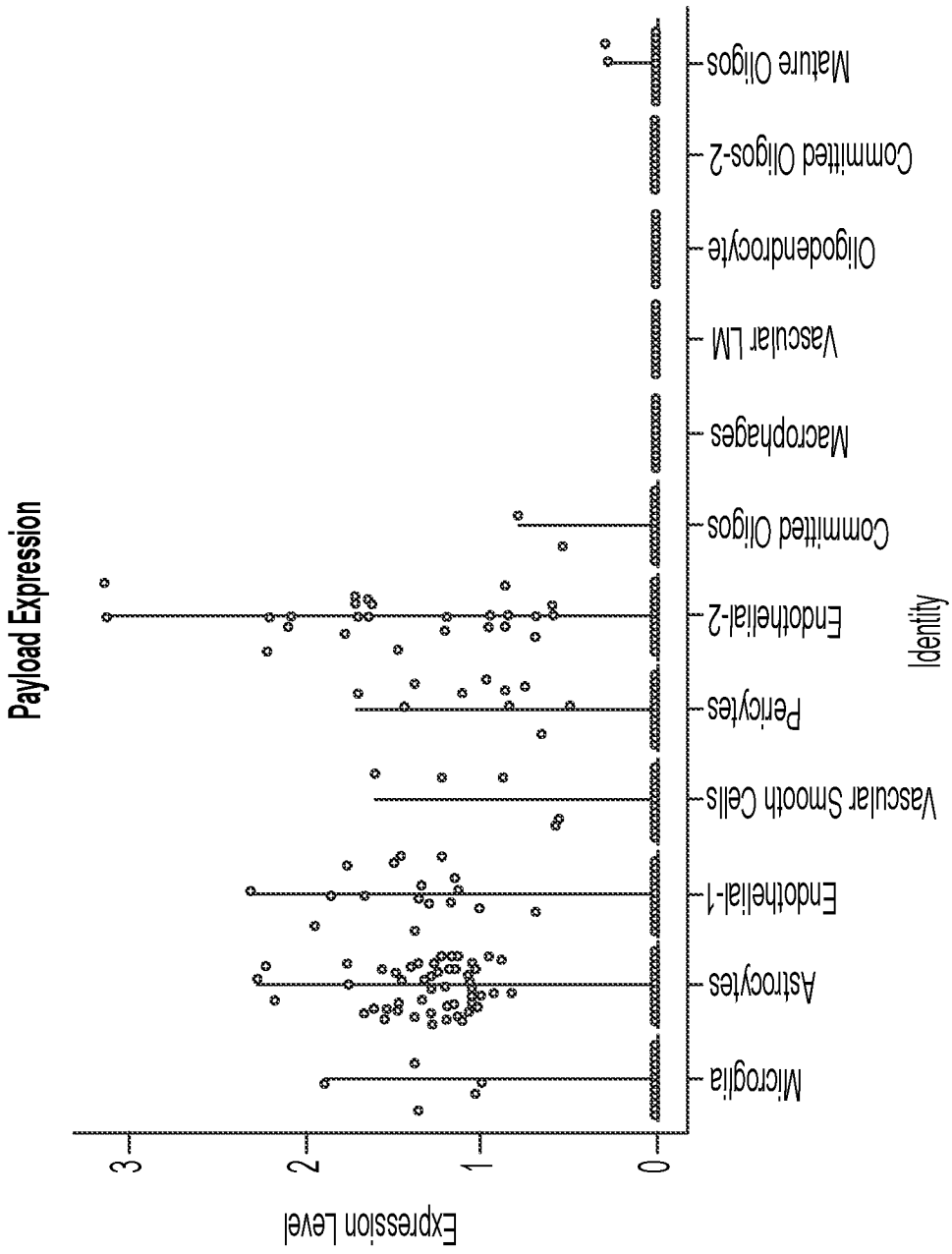
61. The pharmaceutical composition of claim 54, the AAV particle of any one of claims 42-50, an AAV particle comprising the capsid variant of any one of claims 1-37 or 50, or an AAV particle comprising the peptide of any one of claims 39-41 or 50, for use in a method of delivering a payload to a cell or tissue.

62. The pharmaceutical composition of claim 54, the AAV particle of any one of claims 42-50, an AAV particle comprising the capsid variant of any one of claims 1-37 or 50, or an AAV particle comprising the peptide of any one of claims 39-41 or 50, for use in a method of treating a neurological disorder, a neurodegenerative disorder, a muscular disorder, a neuromuscular disorder, or a neuro-oncological disorder.

63. The p pharmaceutical composition of claim 54, the AAV particle of any one of claims 42-50, an AAV particle comprising the capsid variant of any one of claims 1-37 or 50, or an AAV particle comprising the peptide of any one of claims 39-41 or 50, for use in the manufacture of a medicament.

64. Use of the pharmaceutical composition of claim 54, the AAV particle of any one of claims 42-50, an AAV particle comprising the capsid variant of any one of claims 1-37 or 50, or an AAV particle comprising the peptide of any one of claims 39-41 or 50, in the manufacture of a medicament for treating a neurological disorder, a neurodegenerative disorder, a muscular disorder, a neuromuscular disorder, or a neuro-oncological disorder.

65. Use of the pharmaceutical composition of claim 54, the AAV particle of any one of claims 42-50, an AAV particle comprising the capsid variant of any one of claims 1-37 or 50, or an AAV particle comprising the peptide of any one of claims 39-41 or 50, in the manufacture of a medicament.


Payload Expression
Expression Level
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1
2
3
Identity
Microglia
Astrocytes
Endothelial-1
Vascular Smooth Cells
Pericytes
Endothelial-2
Committed Oligos
Macrophages
Vascular LM
Oligodendrocyte
Committed Oligos-2
Mature Oligos

FIG. 1A

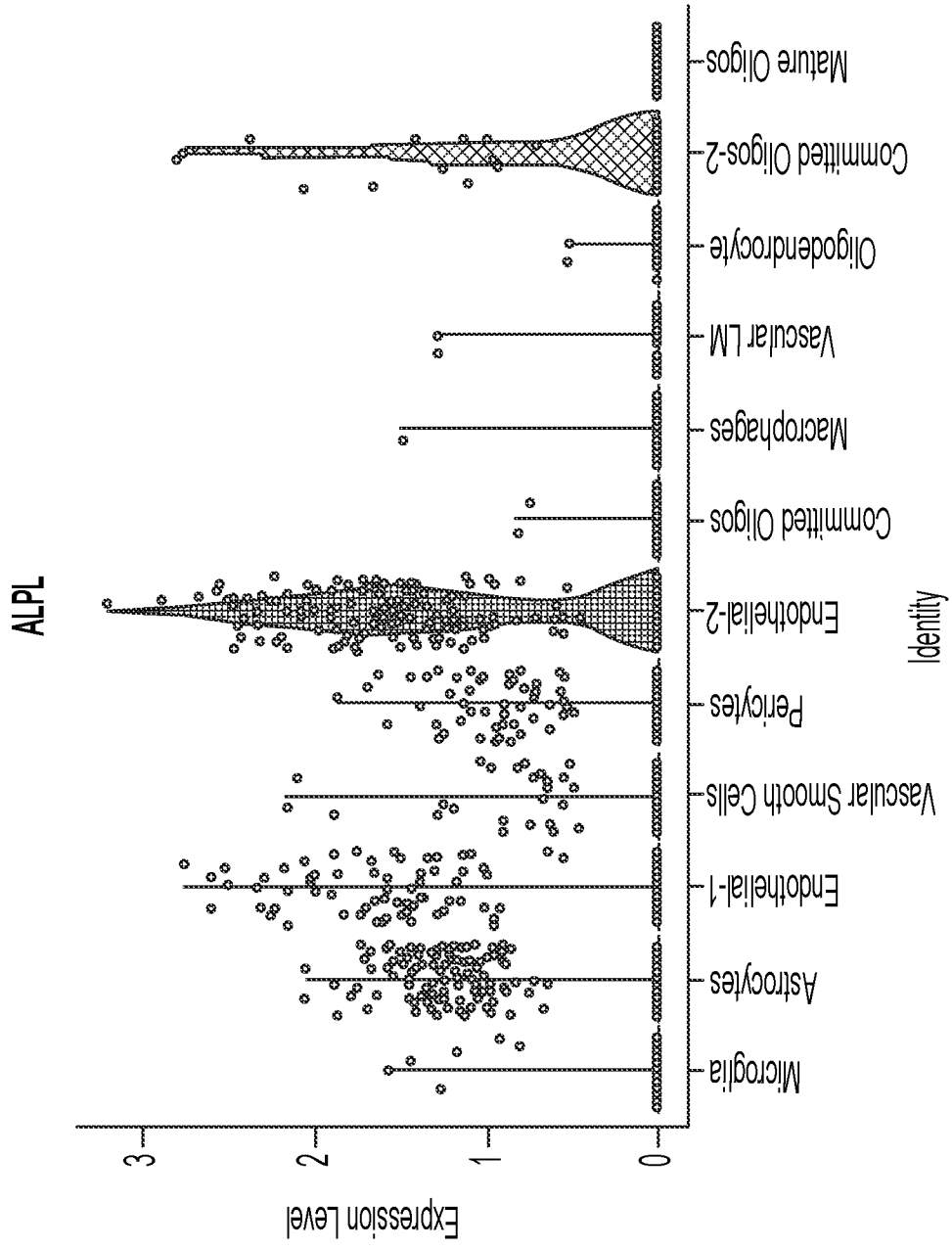
ALPL
Expression Level
0
1
2
3
Identity
Microglia
Astrocytes
Endothelial-1
Vascular Smooth Cells
Pericytes
Endothelial-2
Committed Oligos
Macrophages
Vascular LM
Oligodendrocyte
Committed Oligos-2
Mature Oligos

FIG. 1B

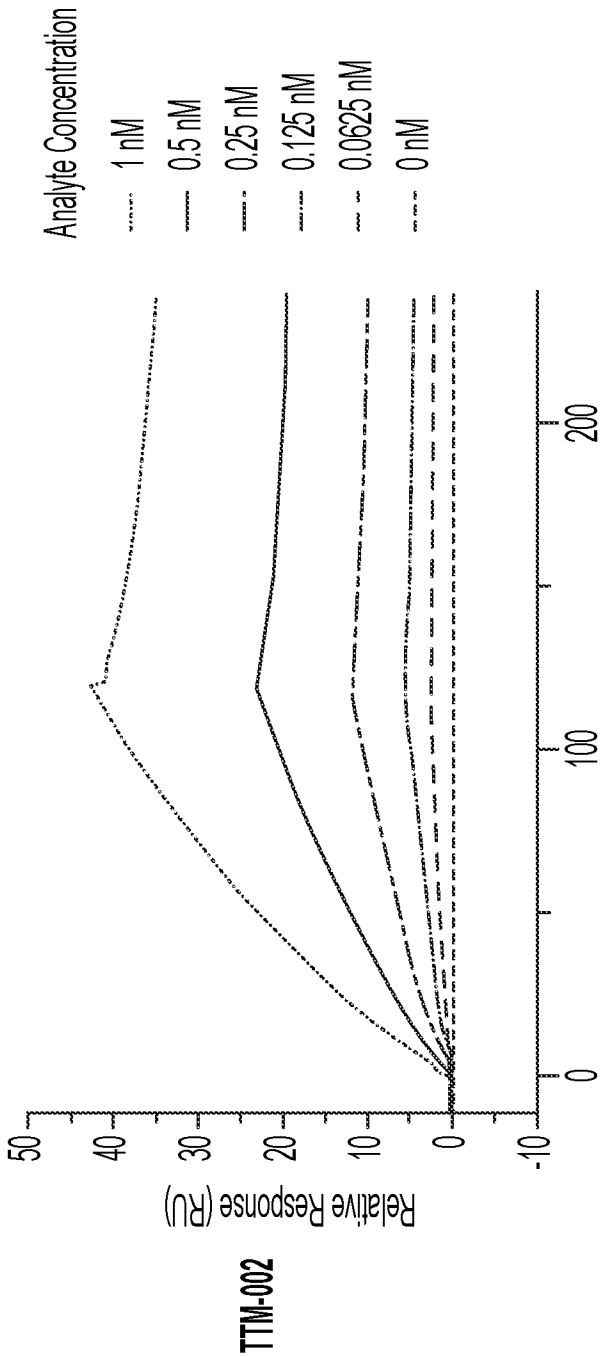
Analyte Concentration
1 nM
0.5 nM
0.25 nM
0.125 nM
0.0625 nM
0 nM
Relative Response (RU)
50
40
30
20
10
0
-10
0
100
200
Time (s)
TTM-002

FIG. 2A

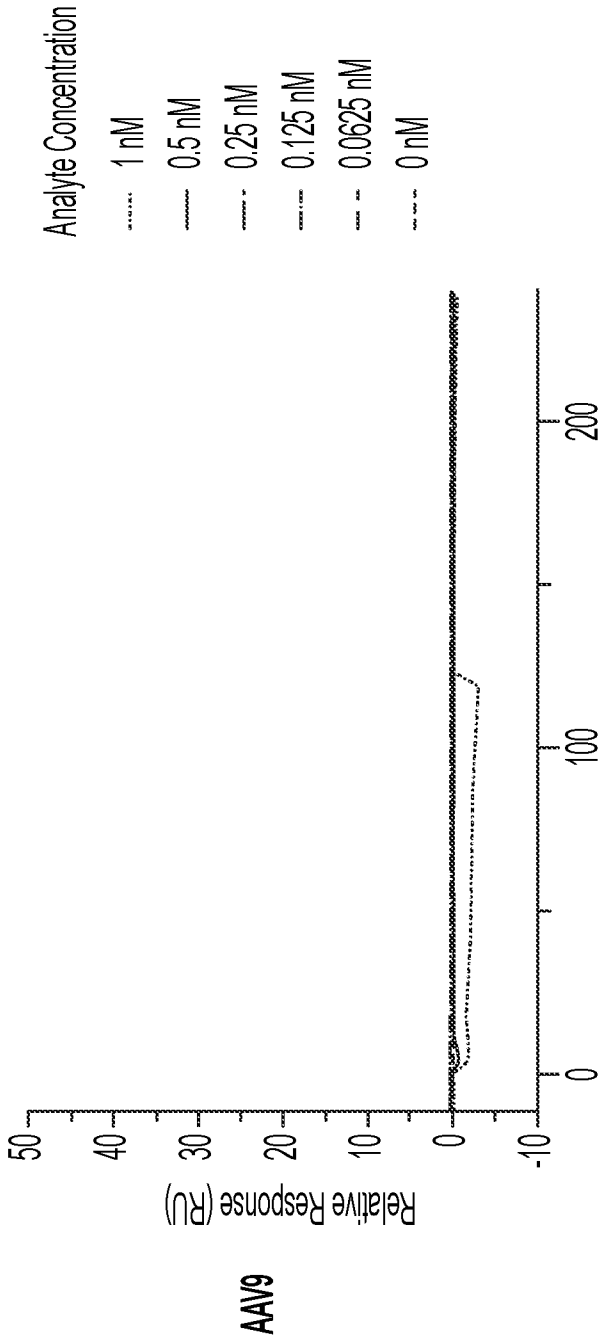
AAV9
Relative Response (RU)
50
40
30
20
10
0
-10
0
100
200
Time (s)
Analyte Concentration
1 nM
0.5 nM
0.25 nM
0.125 nM
0.0625 nM
0 nM

FIG. 2B

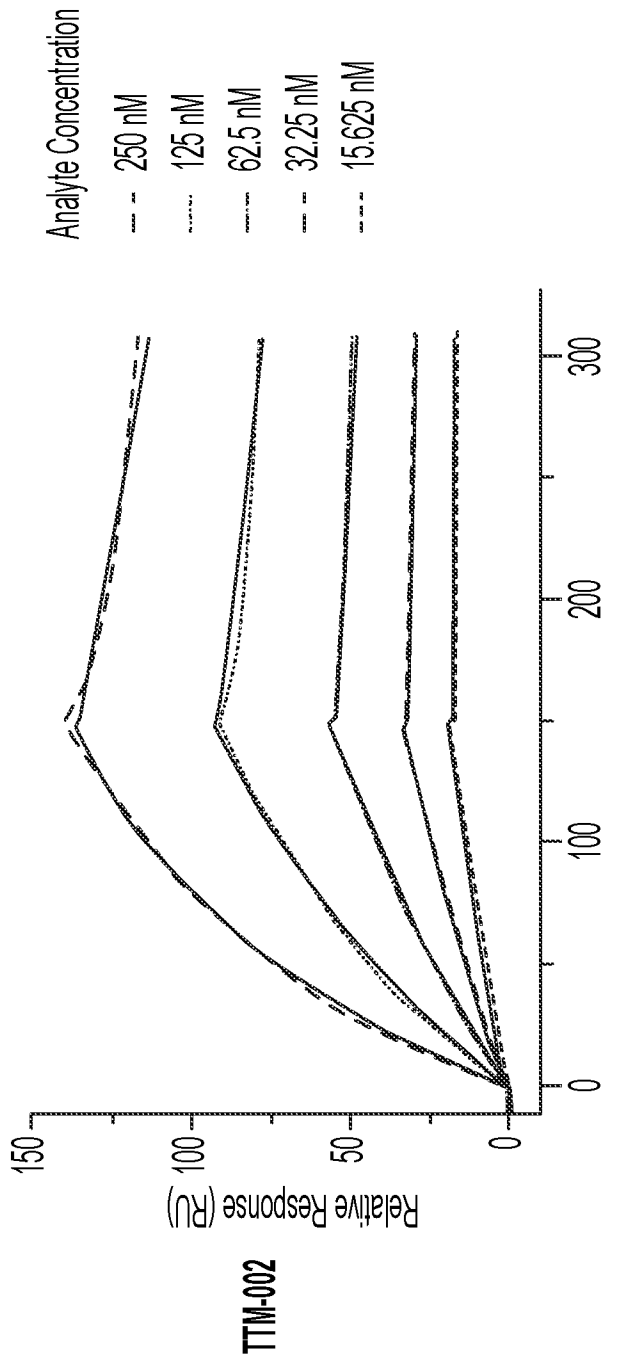
Analyte Concentration
250 nM
125 nM
62.5 nM
32.25 nM
15.625 nM
TTM-002
Relative Response (RU)
150
100
50
0
0
100
200
300
Time (s)

FIG. 2C

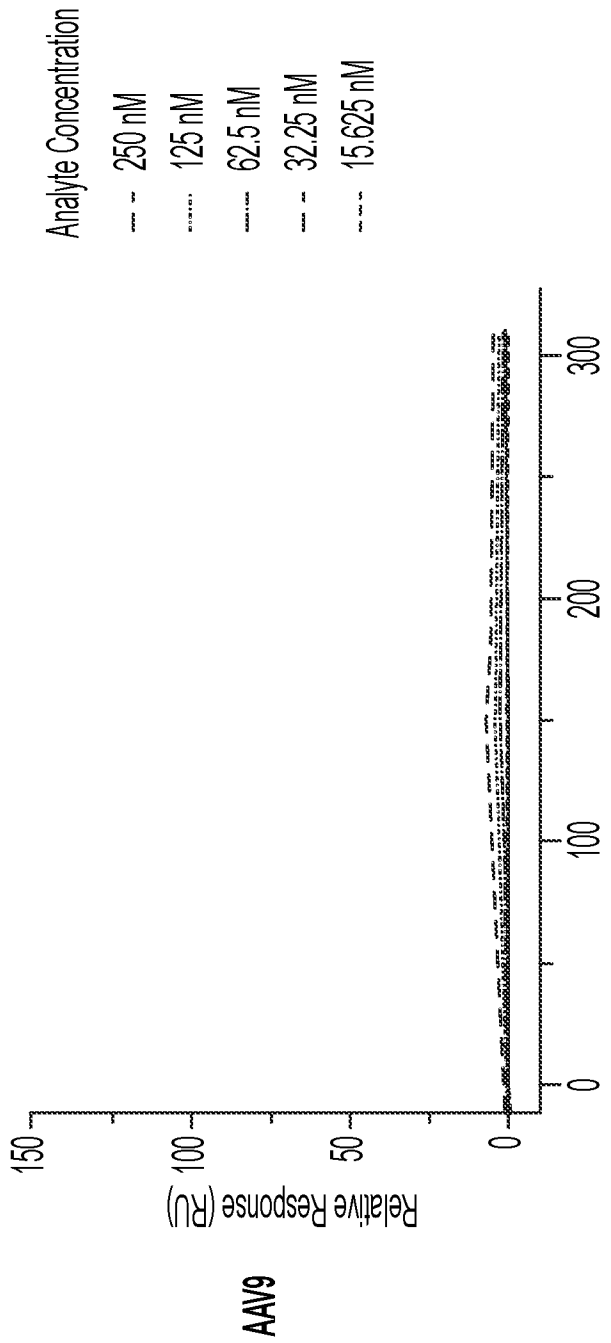
Analyte Concentration
250 nM
125 nM
62.5 nM
32.25 nM
15.625 nM
AAV9
Relative Response (RU)
150
100
50
0
0
100
200
300
Time (s)

FIG. 2D

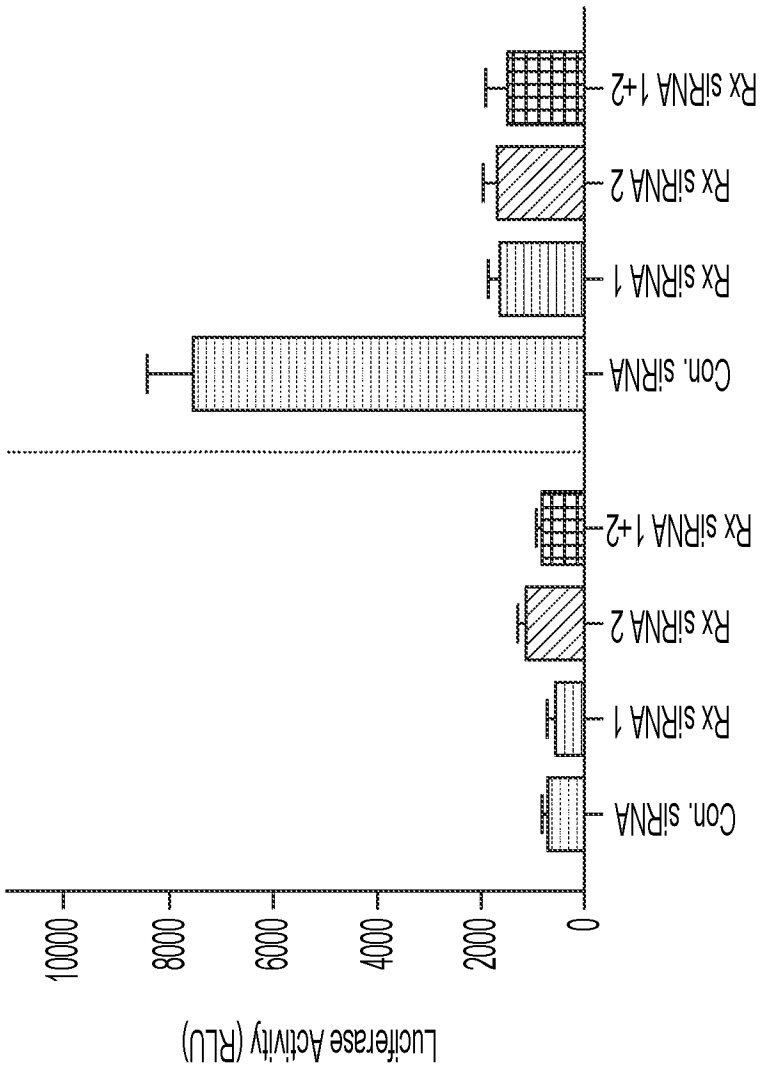
Luciferase Activity (RLU)
10000
8000
6000
4000
2000
0
AAV9
TTM-002
Con. siRNA
Rx siRNA 1
Rx siRNA 2
Rx siRNA 1+2
Con. siRNA
Rx siRNA 1
Rx siRNA 2
Rx siRNA 1+2

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/026263

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K38/00 A61K48/00 C07K14/005 C12N7/00 C12N15/86
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2018/204797 A1 (VOYAGER THERAPEUTICS INC [US]) 8 November 2018 (2018-11-08) See opinion for details	1-65
Y	WO 2022/026409 A1 (VOYAGER THERAPEUTICS INC [US]) 3 February 2022 (2022-02-03) See opinion for details	1-65

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 September 2024	14/10/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Landré, Julien

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/026263

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/026263

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HORDEAUX JULIETTE ET AL: "The GPI-Linked Protein LY6A Drives AAV-PHP.B Transport across the Blood-Brain Barrier", MOLECULAR THERAPY, vol. 27, no. 5, 1 May 2019 (2019-05-01), pages 912-921, XP055950922, US ISSN: 1525-0016, DOI: 10.1016/j.ymthe.2019.02.013 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6520463/pdf/main.pdf> the whole document	1-65

Y	WO 2021/230987 A1 (VOYAGER THERAPEUTICS INC [US]) 18 November 2021 (2021-11-18) the whole document	1-65

X,P	WO 2024/030976 A2 (VOYAGER THERAPEUTICS INC [US]) 8 February 2024 (2024-02-08) the whole document	1-65

X,P	WO 2023/220695 A2 (VOYAGER THERAPEUTICS INC [US]) 16 November 2023 (2023-11-16) the whole document	1-65

X,P	WO 2023/081648 A1 (VOYAGER THERAPEUTICS INC [US]) 11 May 2023 (2023-05-11) the whole document	1-65

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2024/026263

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		KR 20230022175 A	14-02-2023
		PE 20230767 A1	09-05-2023
		TW 202208397 A	01-03-2022
		US 2022042044 A1	10-02-2022
		US 2023203102 A1	29-06-2023
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		WO 2021230987 A1	18-11-2021
WO 2024030976 A2	08-02-2024	NONE	
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		IL 311861 A	01-06-2024
		KR 20240113624 A	22-07-2024
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