

Claims

What is claimed is:

1. A recombinant adeno-associated virus (rAAV) capsid protein, comprising a variant polypeptide sequence at one or more of a VR-IV site, a VR-V site, a VR-VII site, and a VR-VIII site of a parental sequence, wherein the parental sequence comprises a sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 463.
2. The capsid protein of claim 1, wherein the variant polypeptide sequence is a cardiotrophic variant polypeptide sequence.
3. The capsid protein of claim 1 or 2, wherein the capsid protein comprises a variant polypeptide at the VR-IV site of the parental sequence.
4. The capsid protein of claim 3, wherein the variant polypeptide at the VR-IV site has a sequence:

-X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-

wherein:

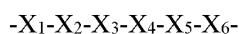
- a) X₁ is G, S or V;
 - b) X₂ is Y, Q or I;
 - c) X₃ is H, W, V or I;
 - d) X₄ is K or N;
 - e) X₅ is S, G or I;
 - f) X₆ is G or R;
 - g) X₇ is A, P or V;
 - h) X₈ is A or R; and
 - i) X₉ is Q or D (SEQ ID NO: 477).
5. The capsid protein of claim 3, wherein the variant polypeptide at the VR-IV site comprises an amino acid sequence selected from SEQ ID NOs: 6-104.
 6. The capsid protein of claim 3, wherein the variant polypeptide at the VR-IV site comprises an amino acid sequence selected from GYHKSGAAQ (SEQ ID NO: 6),

VIIKSGAAQ (SEQ ID NO: 7), GYHKIGAAQ (SEQ ID NO: 8), SQVNGRPRD (SEQ ID NO: 33) and GYHKSGVAQ (SEQ ID NO: 9).

7. The capsid protein of claim 3, wherein the variant polypeptide at the VR-IV site comprises the amino acid sequence GYHKSGAAQ (SEQ ID NO: 6) or a sequence comprising at most 1, 2, 3, or 4 amino-acid substitutions relative to GYHKSGAAQ (SEQ ID NO: 6).

8. The capsid protein of any one of claims 1-7, wherein the capsid protein comprises a variant polypeptide at the VR-V site of the parental sequence.

9. The capsid protein of claim 8, wherein the variant polypeptide at the VR-V site has a sequence:



wherein:

- a) X_1 is S, L, H, N, or A;
- b) X_2 is T, M, K, G, or N;
- c) X_3 is S, T, M or I;
- d) X_4 is S, P, F, M, or N;
- e) X_5 is F, S, P or L; and
- f) X_6 is I, V, or T (SEQ ID NO: 474).

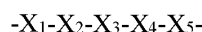
10. The capsid protein of claim 8, wherein the variant polypeptide at the VR-V site comprises an amino acid sequence selected from SEQ ID NOs: 105-203.

11. The capsid protein of claim 8, wherein the variant polypeptide at the VR-V site comprises an amino acid sequence selected from LNSMLI (SEQ ID NO: 105), NGMSFT (SEQ ID NO: 106), HKTF SI (SEQ ID NO: 107) and SMSNFV (SEQ ID NO: 108).

12. The capsid protein of claim 8, wherein the variant polypeptide at the VR-V site comprises the amino acid sequence LNSMLI (SEQ ID NO: 105) or a sequence comprising at most 1, 2, 3, or 4 amino-acid substitutions relative to LNSMLI (SEQ ID NO: 105).

13. The capsid protein of any one of claims 1-12, wherein the capsid protein comprises a variant polypeptide at the VR-VII site of the parental sequence.

14. The capsid protein of claim 13, wherein the variant polypeptide at the VR-VII site has a sequence:



wherein:

- a) X_1 is V, L, Q, C, or R;
- b) X_2 is S, H, G, C, or D;
- c) X_3 is Y, S, L, G, or N;
- d) X_4 is S, L, H, Q, or N; and
- e) X_5 is V, I, or R (SEQ ID NO: 475).

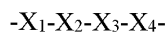
15. The capsid protein of claim 13, wherein the variant polypeptide at the VR-VII site comprises an amino acid sequence selected from SEQ ID NOs: 204-302.

16. The capsid protein of claim 13, wherein the variant polypeptide at the VR-VII site comprises an amino acid sequence selected from RGNQV (SEQ ID NO: 204), VSLNR (SEQ ID NO: 205), CDYSV (SEQ ID NO: 206), and QHGH (SEQ ID NO: 207).

17. The capsid protein of claim 13, wherein the variant polypeptide at the VR-VII site comprises the amino acid sequence RGNQV (SEQ ID NO: 204) or a sequence comprising at most 1, 2, or 3 amino-acid substitutions relative to RGNQV (SEQ ID NO: 204).

18. The capsid protein of any one of claims 1-17, wherein the capsid protein comprises a variant polypeptide at the VR-VII site of the parental sequence.

19. The capsid protein of claim 18, wherein the variant polypeptide at the VR-VIII site has a sequence:



wherein:

- a) X₁ is S, N, or A;
- b) X₂ is V, M, N, or A;
- c) X₃ is Y, V, S, or G; and
- d) X₄ is Y, T, M, G, or N (SEQ ID NO: 476).

20. The capsid protein of claim 18, wherein the variant polypeptide at the VR-VIII site comprises an amino acid sequence selected from SEQ ID NOs: 303-401.
21. The capsid protein of claim 18, wherein the variant polypeptide at the VR-VIII site comprises an amino acid sequence selected from ANYG (SEQ ID NO: 305), NVSY (SEQ ID NO: 303), SMVN (SEQ ID NO: 304), and NVGT (SEQ ID NO: 306).
22. The capsid protein of claim 18, wherein the variant polypeptide at the VR-VIII site comprises the amino acid sequence ANYG (SEQ ID NO: 305) or a sequence comprising at most 1 or 2 amino-acid substitutions relative to ANYG (SEQ ID NO: 305).
23. The capsid protein of claim 18, wherein the variant polypeptide at the VR-VIII site comprises the amino acid sequence NVSY (SEQ ID NO: 303) or a sequence comprising at most 1 or 2 amino-acid substitutions relative to NVSY (SEQ ID NO: 303).
24. The capsid protein of claim 1, wherein the capsid protein comprises an amino acid sequence at least 95% identical to a sequence selected from SEQ ID NOs: 402-410.
25. The capsid protein of claim 1, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 402.
26. The capsid protein of claim 1, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 403.
27. The capsid protein of claim 1, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 404.
28. The capsid protein of claim 1, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 406.

29. The capsid protein of claim 1, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 409.
30. The capsid protein of claim 1, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 483.
31. The capsid protein of any one of claims 1-23, wherein the capsid protein is a AAV5/AAV9 chimeric capsid protein.
32. The capsid protein of claim 31, wherein the capsid protein comprises at least one segment from an AAV5 capsid protein.
33. The capsid protein of claim 31 or 32, wherein the capsid protein comprises:
- a) a first segment comprising a sequence at least 95% identical to SEQ ID NO: 411 or SEQ ID NO: 412;
 - b) a second segment comprising a sequence at least 95% identical to SEQ ID NO: 413 or SEQ ID NO: 414;
 - c) a third segment comprising a sequence at least 95% identical to SEQ ID NO: 415 or SEQ ID NO: 416;
 - d) a fourth segment comprising a sequence at least 95% identical to SEQ ID NO: 417 or SEQ ID NO: 418;
 - e) a fifth segment comprising a sequence at least 95% identical to SEQ ID NO: 419 or SEQ ID NO: 420;
- wherein at least one segment is from an AAV5 capsid protein and at least one segment is from an AAV9 capsid protein.
34. The capsid protein of claim 31, wherein the chimeric capsid protein comprises an amino acid sequence at least 95% identical to a sequence selected from SEQ ID NOs: 445-462.
35. The capsid protein of claim 31, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 457.
36. The capsid protein of claim 31, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 459.

37. The capsid protein of claim 31, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 445.
38. The capsid protein of claim 31, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 446.
39. The capsid protein of claim 31, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 447.
40. The capsid protein of claim 31, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 448.
41. A recombinant adeno-associated virus (rAAV) capsid protein, comprising a sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 463.
42. The capsid protein of claim 41, wherein the variant polypeptide sequence is a cardiotrophic variant polypeptide sequence.
43. The capsid protein of claim 41 or 42, wherein the capsid protein comprises at least one segment from an AAV5 capsid protein.
44. The capsid protein of any one of claims 41-43, wherein the capsid protein comprises:
- f) a first segment comprising a sequence at least 95% identical to SEQ ID NO: 411 or SEQ ID NO: 412;
 - g) a second segment comprising a sequence at least 95% identical to SEQ ID NO: 413 or SEQ ID NO: 414;
 - h) a third segment comprising a sequence at least 95% identical to SEQ ID NO: 415 or SEQ ID NO: 416;
 - i) a fourth segment comprising a sequence at least 95% identical to SEQ ID NO: 417 or SEQ ID NO: 418;
 - j) a fifth segment comprising a sequence at least 95% identical to SEQ ID NO: 419 or SEQ ID NO: 420.

wherein at least one segment is from an AAV5 capsid protein and at least one segment from an AAV9 capsid protein.

45. The capsid protein of any one of claims 41-44, wherein the chimeric capsid protein comprising a sequence at least 95% identical to a sequence selected from SEQ ID NOs: 421-444.
46. The capsid protein of claim 45, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 434.
47. The capsid protein of claim 45, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 438.
48. The capsid protein of claim 45, wherein the capsid protein comprises an amino acid sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 441.
49. A recombinant adeno-associated virus (rAAV) virion, comprising:
a) the capsid protein of any one of claims 1 to 48; and
b) a heterologous nucleic acid comprising a nucleotide sequence encoding one or more gene products.
50. The rAAV virion of claim 49, wherein the rAAV virion exhibits increased transduction efficiency in cardiac cells compared to an AAV virion comprising the parental sequence.
51. The rAAV virion of claim 49 or 50, wherein the rAAV virion exhibits increased transduction efficiency in induced pluripotent stem cell-derived cardiomyocyte (iPS-CM) cells compared to an AAV virion comprising the parental sequence.
52. The rAAV virion of any one of claims 49-51, wherein the rAAV virion exhibits increased transduction efficiency in human cardiac fibroblast (hCF) cells compared to an AAV virion comprising the parental sequence.
53. The rAAV virion of claim 52, wherein the human cardiac fibroblasts are located in the left ventricle of the heart.
54. The rAAV virion of claim 51, wherein the rAAV virion exhibits at least 2-fold increased transduction efficiency in iPS-CM cells at a multiplicity of infection (MOI) of 100,000.

55. The rAAV virion of claim 51, wherein the rAAV virion exhibits at least 2-fold increased transduction efficiency in iPS-CM cells at a multiplicity of infection (MOI) of 75,000.
56. The rAAV virion of any one of claims 49-55, wherein the rAAV virion exhibits at least 2-fold increased cardiac transduction efficiency in a C57BL/6J mouse, wherein the mouse is injected with a virion dosage of 2.5×10^{11} vg/mouse.
57. The rAAV virion of any one of claims 49-55, wherein the rAAV virion exhibits at least 1.5-fold increased cardiac transduction efficiency in a C57BL/6J mouse, wherein the mouse is injected with a virion dosage of 2×10^{11} vg/mouse.
58. The rAAV virion of any one of claims 49-55, wherein the rAAV virion exhibits at least 2-fold increased cardiac transduction efficiency in a C57BL/6J mouse, wherein the mouse is injected with a virion dosage of 1×10^{11} vg/mouse.
59. The rAAV virion of any one of claims 49-58, wherein the rAAV virion exhibits decreased transduction efficiency in liver cells compared to an AAV virion comprising the parental sequence.
60. The rAAV virion of any one of claims 49-59, wherein the rAAV virion exhibits improved NAb evasion compared to an AAV virion comprising the parental sequence.
61. The rAAV virion of any one of claims 49-60, wherein the rAAV virion exhibits increased selectivity of the rAAV virion for cardiac cells over liver cells.
62. The rAAV virion of any one of claims 49-61, wherein the rAAV virion exhibits increased selectivity of the rAAV virion for iPS-CM cells over liver cells.
63. The rAAV virion of any one of claims 49-62, wherein the capsid protein comprises a sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 404.
64. The rAAV virion of any one of claims 49-62, wherein the capsid protein comprises a sequence at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 483.
65. A pharmaceutical composition comprising the rAAV virion of any one of claims 49-64 and a pharmaceutically acceptable carrier.

66. A polynucleotide encoding the capsid protein of any one of claims 1-64.
67. A method of transducing a cardiac cell, comprising contacting the cardiac cell with an rAAV virion according to any one of claims 49-64, wherein the rAAV virion transduces the cardiac cell.
68. The method of claim 67, wherein the cardiac cell is a cardiomyocyte.
69. The method of claim 67 or 68, wherein the rAAV virion exhibits increased transduction efficiency in the cell compared to an AAV virion comprising AAV9 capsid protein sequence.
70. The method of any one of claims 67-69, wherein the rAAV virion exhibits at least 2-fold increased transduction efficiency in the cell compared to an AAV virion comprising AAV9 capsid protein sequence at a multiplicity of infection (MOI) of 75,000.
71. A method of delivering one or more gene products to a cardiac cell, comprising contacting the cardiac cell with an rAAV virion according to any one of claims 49-64.
72. The method of claim 71, wherein the cardiac cell is a cardiomyocyte.
73. A method of treating a cardiac pathology in a subject in need thereof, comprising administering a therapeutically effective amount of a rAAV virion of any one of claims 49-64 or a pharmaceutical composition of claim 61 to the subject, wherein the rAAV virion transduces cardiac tissue.
74. The method of claim 73, wherein the one or more gene products comprise MYBPC3, DWORF, KCNH2, TRPM4, DSG2, PKP2 and/or ATP2A2.
75. The method of claim 73, wherein the one or more gene products comprise CACNA1C, DMD, DMPK, EPG5, EVC, EVC2, FBN1, NF1, SCN5A, SOS1, NPR1, ERBB4, VIP, MYH7, and/or Cas9.
76. The method of claim 73, wherein the one or more gene products comprise MYOCD, ASCL1, GATA4, MEF2C, TBX5, miR-133, and/or MESP1.
77. A kit comprising the pharmaceutical composition of claim 67 and instructions for use.