

CLAIMS

What is claimed is:

- 5 1. A modified adeno-associated virus (AAV) capsid protein comprising an amino acid substitution at one or more positions corresponding to amino acids S194, G474, N564, and/or N573, wherein numbering of the positions is based on VP1 numbering of AAV5.
2. The modified capsid protein of claim 1, wherein numbering of the positions is based on
10 the amino acid sequence of the wild-type AAV5 VP1 as set forth in SEQ ID NO:1.
3. The modified capsid protein of claim 1 or 2, wherein the modified capsid protein is a modified capsid protein of an AAV serotype selected from the group consisting of AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVB1, AAVAnc80, AAV7m8, AAVrh10,
15 AAV2(Y444F), AAV2(Y444+500+730), AAV2(Y252+272+444+500+700+704+730F), AAV8(Y733F), and any variant thereof.
4. The modified capsid protein of claim 3, wherein the modified capsid protein is a modified capsid protein of AAV5.
- 20 5. The modified capsid protein of any one of claims 1 to 4, wherein the modified capsid protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity with the amino acid sequence set forth in
25 SEQ ID NO:1.
6. The modified capsid protein of any one of claims 1 to 5, wherein the modified capsid protein comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence set forth in SEQ ID NO:1.
- 30 7. The modified capsid protein of any one of claims 1 to 6, wherein the modified capsid protein comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence set forth in SEQ ID NO:1.
- 35 8. The modified capsid protein of any one of claims 1 to 3, wherein the amino acid in the capsid protein corresponding to amino acid 194 is G.

9. The modified capsid protein of claim 8, wherein the modified capsid protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity with the amino acid sequence set forth in SEQ ID NO:3, wherein
5 the amino acid in the capsid protein corresponding to amino acid 194 of SEQ ID NO:3 is G.

10. The modified capsid protein of any one of claims 1 to 3, wherein the amino acid in the capsid protein corresponding to amino acid 474 is R.

10 11. The modified capsid protein of claim 10, wherein the modified capsid protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity with the amino acid sequence set forth in SEQ ID NO:5, wherein
15 the amino acid in the capsid protein corresponding to amino acid 474 of SEQ ID NO:5 is R.

12. The modified capsid protein of any one of claims 1 to 3, wherein the amino acid in the capsid protein corresponding to amino acid 564 is R.

13. The modified capsid protein of claim 12, wherein the modified capsid protein comprises
20 an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity with the amino acid sequence set forth in SEQ ID NO:7, wherein the amino acid in the capsid protein corresponding to amino acid 564 of SEQ ID NO:7 is R.

14. The modified capsid protein of any one of claims 1 to 3, wherein the amino acid in the capsid protein corresponding to amino acid 573 is R.

15. The modified capsid protein of claim 14, wherein the modified capsid protein comprises
30 an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity with the amino acid sequence set forth in SEQ ID NO:9, wherein the amino acid in the capsid protein corresponding to amino acid 573 of SEQ ID NO:9 is R.

16. A modified adeno-associated virus (AAV) capsid protein comprising:
35 a G at the position corresponding to amino acid 194;
an R at the position corresponding to amino acid 474;
an R at the position corresponding to amino acid 564; and/or

an R at the position corresponding to amino acid 573, wherein numbering of the position is based on VP1 numbering of AAV5.

17. A modified adeno-associated virus (AAV) capsid protein comprising a G at the position
5 corresponding to amino acid 194, wherein numbering of the position is based on VP1 numbering of AAV5.

18. A modified adeno-associated virus (AAV) capsid protein comprising an R at the position corresponding to amino acid 474, wherein numbering of the position is based on VP1
10 numbering of AAV5.

19. A modified adeno-associated virus (AAV) capsid protein comprising an R at the position corresponding to amino acid 564, wherein numbering of the position is based on VP1 numbering of AAV5.
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20. A modified adeno-associated virus (AAV) capsid protein comprising an R at the position corresponding to amino acid 573, wherein numbering of the position is based on VP1 numbering of AAV5.

20 21. A modified adeno-associated virus (AAV) capsid protein comprising the amino acid sequence set forth in SEQ ID NOs:3, 5, 7, or 9.

22. An isolated nucleic acid encoding the capsid protein of any one of claims 1 to 21.

25 23. An isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NOs:4, 6, 8, or 10.

24. A vector comprising the nucleic acid of claim 22 or 23.

30 25. The vector of claim 24, wherein the vector is a plasmid or a helper viral vector.

26. The vector of claim 25, wherein the helper viral vector is a retrovirus vector, a herpes virus vector, a baculovirus vector, or an adenovirus vector.

35 27. The vector of any one of claims 24 to 26, wherein the vector is an expression vector.

28. A recombinant cell comprising the nucleic acid of claim 22 or 23, or the vector of any one of claims 24 to 27.

29. A method of producing an AAV capsid protein, the method comprising culturing the recombinant cell of claim 28 under conditions whereby the nucleic acid is expressed and the capsid protein is produced.

- 5 30. A recombinant adeno-associated viral (rAAV) particle comprising:
- (a) an rAAV capsid comprising a modified capsid protein, wherein the modified capsid protein comprises an amino acid substitution at one or more positions corresponding to amino acids 194, 474, 564, and/or 573, wherein numbering of the positions is based on VP1 numbering of AAV5; and
- 10 (b) an rAAV vector comprising a heterologous nucleic acid.

31. The rAAV particle of claim 30, wherein numbering of the positions is based on the amino acid sequence of the wild-type AAV5 VP1 as set forth in SEQ ID NO:1.

- 15 32. The rAAV particle of claim 30 or 31, wherein the modified capsid protein is a modified capsid protein of an AAV serotype selected from the group consisting of AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVB1, AAVAnc80, AAV7m8, AAVrh10, AAV2(Y444F), AAV2(Y444+500+730), AAV2(Y252+272+444+500+700+704+730F), AAV8(Y733F), and any variant thereof.

- 20 33. The rAAV particle of any one of claims 30 to 32, wherein the modified capsid protein is a modified capsid protein of AAV5.

34. The rAAV particle of any one of claims 30 to 33, wherein the modified capsid protein
- 25 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity with the amino acid sequence set forth in SEQ ID NO:1.

- 30 35. The rAAV particle of any one of claims 30 to 34, wherein the modified capsid protein comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence set forth in SEQ ID NO:1.

36. The rAAV particle of any one of claims 30 to 35, wherein the modified capsid protein
- 35 comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence set forth in SEQ ID NO:1.

37. The rAAV particle of any one of claims 30 to 32, wherein the amino acid in the capsid protein corresponding to amino acid 194 is G.

5 38. The rAAV particle of claim 37, wherein the modified capsid protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity with the amino acid sequence set forth in SEQ ID NO:3, wherein the amino acid in the capsid protein corresponding to amino acid 194 of SEQ ID NO:3 is G.

10 39. The rAAV particle of any one of claims 30 to 32, wherein the amino acid in the capsid protein corresponding to amino acid 474 is R.

40. The rAAV particle of claim 39, wherein the modified capsid protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity with the amino acid sequence set forth in SEQ ID NO:5, wherein the amino acid in the capsid protein corresponding to amino acid 474 of SEQ ID NO:5 is R.

20 41. The rAAV particle of any one of claims 30 to 32, wherein the amino acid in the capsid protein corresponding to amino acid 564 is R.

42. The rAAV particle of claim 41, wherein the modified capsid protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity with the amino acid sequence set forth in SEQ ID NO:7, wherein the amino acid in the capsid protein corresponding to amino acid 564 of SEQ ID NO:7 is R.

30 43. The rAAV particle of any one of claims 30 to 32, wherein the amino acid in the capsid protein corresponding to amino acid 573 is R.

44. The rAAV particle of claim 43, wherein the modified capsid protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity with the amino acid sequence set forth in SEQ ID NO:9, wherein the amino acid in the capsid protein corresponding to amino acid 573 of SEQ ID NO:9 is R.

45. A recombinant adeno-associated viral (rAAV) particle comprising:

- (a) an rAAV capsid comprising a modified capsid protein, wherein the modified capsid protein comprises: a G at the position corresponding to amino acid 194, an R at the position corresponding to amino acid 474, an R at the position corresponding to amino acid 564, and/or an R at the position corresponding to amino acid 573, wherein
5 numbering of the position is based on VP1 numbering of AAV5; and
(b) an rAAV vector comprising a heterologous nucleic acid.

46. A recombinant adeno-associated viral (rAAV) particle comprising:

- (a) an rAAV capsid comprising a G at the position corresponding to amino acid 194,
10 wherein numbering of the position is based on VP1 numbering of AAV5; and
(b) an rAAV vector comprising a heterologous nucleic acid.

47. A recombinant adeno-associated viral (rAAV) particle comprising:

- (a) an rAAV capsid comprising an R at the position corresponding to amino acid 474,
15 wherein numbering of the position is based on VP1 numbering of AAV5; and
(b) an rAAV vector comprising a heterologous nucleic acid.

48. A recombinant adeno-associated viral (rAAV) particle comprising:

- (a) an rAAV capsid comprising an R at the position corresponding to amino acid 564,
20 wherein numbering of the position is based on VP1 numbering of AAV5; and
(b) an rAAV vector comprising a heterologous nucleic acid.

49. A recombinant adeno-associated viral (rAAV) particle comprising:

- (a) an rAAV capsid comprising an R at the position corresponding to amino acid 573,
25 wherein numbering of the position is based on VP1 numbering of AAV5; and
(b) an rAAV vector comprising a heterologous nucleic acid.

50. The rAAV particle of any one of claims 30 to 49, wherein the heterologous nucleic acid encodes a therapeutic polypeptide or therapeutic nucleic acid.

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51. The rAAV particle of claim 50, wherein the heterologous nucleic acid encodes a polypeptide selected from the group consisting of an antioxidant, an enzyme, a neurotrophic factor, an anti-apoptotic factor, an anti-angiogenic factor, and an anti-inflammatory factor.

35 52. The rAAV particle of any one of claims 30 to 49, wherein the heterologous nucleic acid encodes a therapeutic nucleic acid.

53. The rAAV particle of claim 52, wherein the therapeutic nucleic acid is an siRNA, an shRNA, an RNAi, an miRNA, an antisense RNA, a ribozyme or a DNzyme.

54. The rAAV particle of any one of claims 30 to 53, wherein the heterologous nucleic acid
5 is operably linked to a constitutive promoter.

55. The rAAV particle of any one of claims 30 to 53, wherein the heterologous nucleic acid is operably linked to a promoter suitable for expression of the therapeutic polypeptide or therapeutic nucleic acid in an ocular tissue.

10 56. The rAAV particle of claim 55, wherein the ocular tissue is a retina, and the promoter is suitable for expression of the therapeutic polypeptide or therapeutic nucleic acid in a retinal cell selected from the group consisting of a photoreceptor cell, a retinal pigmented epithelial cell, a bipolar cell, a horizontal cell, an amacrine cell, a muller cell, a ganglion cell, and any
15 combination thereof.

57. The rAAV particle of claim 56, wherein the ocular tissue is a cornea, and the promoter is suitable for expression of the therapeutic polypeptide or therapeutic nucleic acid in a corneal cell selected from the group consisting of an epithelial cell, a keratocyte, an endothelial cell,
20 and any combination thereof.

58. The rAAV particle of any one of claims 30 to 57, wherein the AAV vector further comprises inverted terminal repeats (ITRs).

25 59. The rAAV particle of any one of claims 30 to 58, wherein the rAAV vector is a self-complementary rAAV vector (scAAV).

60. The rAAV particle of claim 59, wherein the scAAV comprises a first nucleic acid encoding the heterologous nucleic acid, and a second nucleic acid encoding a complement of
30 the first nucleic acid, wherein the first nucleic acid can form intrastrand base pairs with the second nucleic acid along most or all of its length.

61. The rAAV particle of claim 60, wherein the first nucleic acid and the second nucleic acid are linked by a mutated AAV ITR, wherein the mutated AAV ITR comprises a deletion of
35 the D region and comprises a mutation of the terminal resolution sequence.

62. A pharmaceutical composition comprising a rAAV particle of any one of claims 30 to 61.

63. A method for delivering a heterologous nucleic acid to an ocular tissue of a subject in need thereof, comprising administering a recombinant adeno-associated virus (rAAV) particle to the subject, wherein the rAAV particle comprises:

- 5 (a) an rAAV capsid comprising a modified capsid protein, wherein the modified capsid protein comprises an amino acid substitution at one or more positions corresponding to amino acids 194, 474, 564, and/or 573, wherein numbering of the positions is based on VP1 numbering of AAV5; and
- (b) an rAAV vector comprising a heterologous nucleic acid.

10 64. A method for delivering a heterologous nucleic acid to the retina of a subject in need thereof, comprising administering a recombinant adeno-associated virus (rAAV) particle to the subject, wherein the rAAV particle comprises:

- 15 (a) an rAAV capsid comprising a modified capsid protein, wherein the modified capsid protein comprises an amino acid substitution at a position corresponding to amino acid 194, wherein numbering of the positions is based on VP1 numbering of AAV5; and
- (b) an rAAV vector comprising a heterologous nucleic acid.

20 65. A method for delivering a heterologous nucleic acid to the cornea of a subject in need thereof, comprising administering a recombinant adeno-associated virus (rAAV) particle to the subject, wherein the rAAV particle comprises:

- (a) an rAAV capsid comprising a modified capsid protein, wherein the modified capsid protein comprises an amino acid substitution at one or more positions corresponding to amino acids 474, 564, and/or 573, wherein numbering of the positions is based on VP1 numbering of AAV5; and
- 25 (b) an rAAV vector comprising a heterologous nucleic acid.

66. A method for improving rAAV transduction of cells in an ocular tissue of a subject in need thereof, the method comprising administering a recombinant adeno-associated virus (rAAV) particle to the subject, wherein the rAAV particle comprises:

- 30 (a) an rAAV capsid comprising a modified capsid protein, wherein the modified capsid protein comprises an amino acid substitution at one or more positions corresponding to amino acids 194, 474, 564, and/or 573, wherein numbering of the positions is based on VP1 numbering of AAV5; and
- (b) an rAAV vector comprising a heterologous nucleic acid.

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67. A method for improving rAAV transduction of cells in the retina of a subject in need thereof, the method comprising administering a recombinant adeno-associated virus (rAAV) particle to the subject, wherein the rAAV particle comprises

- 5 (a) an rAAV capsid comprising a modified capsid protein, wherein the modified capsid protein comprises an amino acid substitution at a position corresponding to amino acid 194, wherein numbering of the positions is based on VP1 numbering of AAV5; and
- (b) an rAAV vector comprising a heterologous nucleic acid.

68. A method for improving rAAV transduction of cells in the cornea of a subject in need thereof, the method comprising administering a recombinant adeno-associated virus (rAAV) particle to the subject, wherein the rAAV particle comprises

- 10 (a) an rAAV capsid comprising a modified capsid protein, wherein the modified capsid protein comprises an amino acid substitution at one or more positions corresponding to amino acids 474, 564, and/or 573, wherein numbering of the positions is based on
- 15 VP1 numbering of AAV5; and
- (b) an rAAV vector comprising a heterologous nucleic acid.

69. A method for improving expression of a heterologous nucleic acid in an ocular tissue of a subject in need thereof, the method comprising administering a recombinant adeno-associated virus (rAAV) particle to the subject, wherein the rAAV particle comprises

- 20 (a) an rAAV capsid comprising a modified capsid protein, wherein the modified capsid protein comprises an amino acid substitution at one or more positions corresponding to amino acids 194, 474, 564, and/or 573, wherein numbering of the positions is based on VP1 numbering of AAV5; and
- 25 (b) an rAAV vector comprising a heterologous nucleic acid.

70. A method for improving expression of a heterologous nucleic acid in the retina of a subject in need thereof, the method comprising administering a recombinant adeno-associated virus (rAAV) particle to the subject, wherein the rAAV particle comprises

- 30 (a) an rAAV capsid comprising a modified capsid protein, wherein the modified capsid protein comprises an amino acid substitution at a position corresponding to amino acid 194, wherein numbering of the positions is based on VP1 numbering of AAV5; and
- (b) an rAAV vector comprising a heterologous nucleic acid.

35 71. A method for improving expression of a heterologous nucleic acid in the cornea of a subject in need thereof, the method comprising administering a recombinant adeno-associated virus (rAAV) particle to the subject, wherein the rAAV particle comprises

- (a) an rAAV capsid comprising a modified capsid protein, wherein the modified capsid protein comprises an amino acid substitution at one or more positions corresponding to amino acids 474, 564, and/or 573, wherein numbering of the positions is based on VP1 numbering of AAV5; and
- 5 (b) an rAAV vector comprising a heterologous nucleic acid.

72. A method to treat a condition or a disorder of the eye in a subject in need thereof, the method comprising administering an effective amount of a composition comprising an rAAV particle to the subject, wherein the rAAV particle comprises

- 10 (a) an rAAV capsid comprising a modified capsid protein, wherein the modified capsid protein comprises an amino acid substitution at one or more positions corresponding to amino acids 194, 474, 564, and/or 573, wherein numbering of the positions is based on VP1 numbering of AAV5; and
- (b) an rAAV vector comprising a heterologous nucleic acid.

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73. The method of claim 72, wherein the composition is formulated for intravitreal administration.

74. The method of any one of claims 63 to 73, wherein the heterologous nucleic acid is
- 20 expressed at an increased level of expression, as compared to the level of expression of a heterologous nucleic acid of an rAAV particle comprising a wild-type rAAV capsid.

75. The method of any one of claims 63 to 74, wherein the administering comprises intravitreal administration.

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