

CLAIMS:

We claim:

1. An ~~recombinant~~ AAV vector comprising an AAV capsid protein ~~variant, wherein the capsid protein variant comprises a peptide having~~comprising the sequence of any one of SEQ ID NOs: 2-19.
- ~~2. A recombinant AAV vector comprising an AAV capsid protein variant, wherein the AAV capsid variant has at least 90% identity to the sequence of SEQ ID NO: 1, wherein the amino acids corresponding to amino acids 452-458 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 20-28.~~
- ~~3. A recombinant AAV vector comprising an AAV capsid protein variant, wherein the AAV capsid variant has at least 90% identity to the sequence of SEQ ID NO: 1, wherein the amino acids corresponding to amino acids 586-592 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 29-37.~~
- ~~4. A recombinant AAV vector comprising an AAV capsid protein variant, wherein the AAV capsid variant has at least 90% identity to the sequence of SEQ ID NO: 1,
 - a. wherein the amino acids corresponding to amino acids 452-458 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 20-28; and
 - b. wherein the amino acids corresponding to amino acids 586-592 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 29-37.~~
- ~~5. A recombinant AAV vector comprising an AAV capsid protein variant, wherein the AAV capsid variant has the sequence of any one of SEQ ID NO: 2-19, 46-123 or a sequence with at least 90% or at least 95% identity thereto.~~
- ~~6. A recombinant AAV vector comprising an AAV capsid protein variant, wherein the AAV capsid variant has the sequence of any one of SEQ ID NO: 2-19, 46-123 or a sequence with 1-10, 11-20, 20-30, or 30-50 amino acid substitutions relative thereto.~~

~~7.2.~~ The ~~recombinant~~ AAV vector of ~~any one of claims 1-6~~ claim 1, wherein the AAV vector comprises a vector genome encapsidated by an AAV capsid comprising the AAV capsid protein.

~~8.~~ The ~~recombinant AAV vector of claim 7, wherein the vector genome is encapsidated by an AAV capsid comprising the AAV capsid protein variant.~~

~~9.3.~~ The ~~recombinant~~ AAV vector of claim ~~7 or 8~~ 2, wherein the vector genome comprises a first inverted terminal repeat (ITR) and a second ITR.

~~10.4.~~ The ~~recombinant~~ AAV vector of claim ~~9~~ 3, wherein the vector genome comprises a transgene located between the first ITR and the second ITR.

~~11.5.~~ The ~~recombinant~~ AAV vector of claim ~~10~~ 4, wherein the transgene encodes a therapeutic RNA, a therapeutic protein, or a therapeutic polypeptide.

~~12.~~ The ~~recombinant AAV vector of claim 10, wherein the transgene encodes a therapeutic protein.~~

~~13.6.~~ The ~~recombinant~~ AAV vector of claim ~~10~~ 4, wherein the transgene encodes a gene-editing molecule.

~~14.7.~~ The ~~recombinant~~ AAV vector of claim ~~13~~ 6, wherein the gene-editing molecule is a nuclease.

~~15.8.~~ The ~~recombinant~~ AAV vector of claim ~~14~~ 7, wherein the nuclease is a Cas9 nuclease or a Cas12a nuclease.

~~16.~~ The ~~recombinant AAV vector of claim 14, wherein the nuclease is a Cas12a nuclease.~~

~~17.9.~~ The ~~recombinant~~ AAV vector of claim ~~13~~ 6, wherein the gene-editing molecule is a single guide RNA (sgRNA).

~~18.10.~~ An AAV capsid protein ~~variant~~ comprising ~~a peptide having~~ the sequence of any one of SEQ ID NOs: 2-19.

~~19. An AAV capsid protein variant having at least 90% identity to the sequence of SEQ ID NO: 1, wherein the amino acids corresponding to amino acids 452-458 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 20-28.~~

~~20. An AAV capsid protein variant having at least 90% identity to the sequence of SEQ ID NO: 1, wherein the amino acids corresponding to amino acids 586-592 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 29-37.~~

~~21. An AAV capsid protein variant having at least 90% identity to the sequence of SEQ ID NO: 1,~~

~~a. wherein the amino acids corresponding to amino acids 452-458 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 20-28; and~~

~~b. wherein the amino acids corresponding to amino acids 586-592 of SEQ ID NO: 1 are substituted with a peptide having a sequence of any one of SEQ ID NOs: 29-37.~~

~~22. An AAV capsid protein variant having the sequence of any one of SEQ ID NO: 2-19, 46-123 or a sequence with at least 90% or at least 95% identity thereto.~~

~~23. An AAV capsid protein variant having the sequence of any one of SEQ ID NO: 2-19, 46-123 or a sequence with 1-10, 11-20, 20-30, or 30-50 amino acid substitutions relative thereto.~~

~~24. An AAV capsid comprising the AAV capsid protein variant of any one of claims 18-23.~~

~~25. The AAV capsid of claim 24, wherein the AAV capsid comprises about 60 copies of the AAV capsid protein variant, or fragments thereof.~~

~~26. — The AAV capsid of claim 25, wherein the AAV capsid protein variants are arranged with T=1 icosahedral symmetry.~~

~~27. — A recombinant AAV vector comprising the AAV capsid variant of any one of claims 18-23, or the AAV capsid of any one of claims 24-26.~~

~~28. — A pharmaceutical composition comprising the recombinant AAV vector of any one of claims 1-17, and 27, and at least one pharmaceutically acceptable carrier.~~

~~29~~11. A method of introducing a ~~recombinant AAV vector~~transgene into a ~~target~~ cell, the method comprising: contacting ~~a target~~the cell with ~~a recombinant~~the AAV vector of any one of claims ~~4-9~~1-17, and 27, or the pharmaceutical composition of claim 28.

~~30~~12. A method of delivering a transgene to a target cell in a subject, the method comprising administering to the subject a ~~recombinant~~Use of the AAV vector of any one of claims ~~1-17, and 27, or the pharmaceutical composition of claim 28~~4-9 in the manufacture of a medicament for delivering a transgene to a cell in a subject.

~~31~~13. The method of ~~any one of claims 29 and 30~~claim 11 or use of claim 12, wherein the ~~target~~ cell is a kidney cell.

~~32. — A method of evolving novel strains of adeno-associated viruses comprising passaging AAV libraries across multiple mammalian species.~~

~~33. — The method according to claim 32, wherein said AAV libraries comprise a plurality of recombinant AAV vectors, wherein each recombinant AAV vector comprises a capsid protein variant comprising one or more amino acid mutations relative to a wildtype AAV capsid protein.~~

~~34. — The method according to claim 33, wherein each recombinant AAV vector in the AAV libraries comprises one or more amino acid mutations relative to a wildtype AAV9 capsid protein (SEQ ID NO: 1).~~

~~35.—The method according to claim 34, wherein the one or more amino acid mutations are in the regions corresponding to amino acids 452-458 of SEQ ID NO: 1 or 586-592 of SEQ ID NO: 1, or the mutations are found in both regions corresponding to amino acids 452-458 and 586-592 of SEQ ID NO: 1.~~

~~36.—The method according to any one of claims 31-35, wherein the method comprises administering a first AAV library to a first mammalian species.~~

~~37.—The method according to claim 36, wherein AAVs from the first AAV library present in one or more target tissues of the first mammalian species are sequenced, and used to generate a second AAV library.~~

~~38.—The method according to claim 37, wherein the second AAV library is administered to a second mammalian species, wherein the first mammalian species and the second mammalian species are different.~~

~~39.—The method according to claim 38, wherein the AAVs from the second AAV library present in one or more target tissues of the second mammalian species and sequenced.~~

~~40.—The method according to any one of claims 36-39, wherein the first mammalian species and the second mammalian species are each independently selected from the group consisting of: *Mus Musculus* (mouse), *Sus scrofa* (pig), *Canis Familiaris* (Dog), Non-human primates (*Macaca*, macaque), and *Homo sapiens* (human).~~

~~41.14. The method according to claim 40 or the use according to claim 12, wherein the one or more target tissues of the first mammalian species cell is selected from in the spinal cord, dorsal root ganglion, brain, heart, lung, kidney, skeletal muscle, spleen, pancreas, small intestine, large intestine, or liver tissue of the subject, and any combination thereof.~~

~~42.—The method according to claim 40, wherein the one or more target tissues of the second mammalian species is selected from spinal cord, dorsal root ganglion, brain, heart, lung, kidney, skeletal muscle, spleen, pancreas, small intestine, large intestine, or liver tissue, and any combination thereof.~~

~~43. A recombinant adeno-associated virus (AAV) comprising a capsid protein variant evolved using the method of any one of claims 31-42.~~

~~44. The recombinant AAV of claim 43, wherein the AAV has improved gene transfer efficiency in one or more mammalian species relative to a recombinant AAV that has a capsid protein that is otherwise identical, except it lacks the one or more amino acid substitutions.~~

~~45. The recombinant AAV of claim 44, wherein the improved gene transfer efficiency is occurs in one more of: *Mus Musculus* (mouse), *Sus scrofa* (pig), *Canis Familiaris* (Dog), Non-human primates (*Macaca*, macaque), or *Homo sapiens* (human).~~

~~46. The recombinant AAV of any one of claims 43-45, wherein the improved gene transfer efficiency occurs in one or more of the following cell types or tissues: spinal cord, dorsal root ganglion, brain, heart, lung, kidney, skeletal muscle, spleen, pancreas, small intestine, large intestine, or liver.~~

~~47. The recombinant AAV of claim 46, wherein the improved gene transfer efficiency occurs in kidney cells or kidney tissue.~~

~~48. A method of treating a subject in need thereof, comprising: administering to the subject an effective amount of the recombinant AAV vector or any one of claims 1-17, 27, and 43-47 or the pharmaceutical composition of claim 28.~~

~~49~~15. The ~~method~~use of claim ~~48~~12, wherein the subject has a kidney disease or kidney disorder.

16. The use of claim 12, wherein the subject is human.

17. The AAV vector of claim 4, further comprising a promoter operably linked to the transgene.

18. A plasmid comprising an AAV cap gene encoding the AAV capsid protein of claim 10.

19. The plasmid of claim 18, further comprising an AAV rep gene.

20. A cell line that stably expresses the AAV capsid protein of claim 10.

21. The cell line of claim 20, further expressing an AAV rep gene.