CLAIMS

1. An extracellular vesicle (EV) comprising an adeno-associated virus (AAV) and a scaffold protein, wherein the AAV is in the lumen of the EV, and wherein the AAV in the EV has altered properties as compared to the AAV alone.

2. The EV of claim 1, wherein

(i) the altered property comprises a better therapeutic effect than AAV alone, wherein the better therapeutic effect comprises one or more of higher infectivity, higher activity, greater potency, faster transduction kinetics, and tolerance against immune invasion;

(ii) the altered property allows the AAV to be administered to a subject through two or more doses, wherein the infectivity and/or activity of the AAV is retained in subsequent doses;

(iii) both (i) and (ii).

3. An EV comprising an AAV, wherein the EV comprises a scaffold protein and at least 5 AAV, wherein the at least 5 AAV are in the lumen of the EV.

4. The EV of claim 3, comprising at least about 5 AAVs to at least about 100 AAVs in the lumen of the EV.

5. The EV of claim 3 or 4 comprising at least about 5 AAVs to at least about 20 AAVs.

6. The EV of any one of claims 1 to 5, wherein the EV comprises a bi-lipid membrane comprising a luminal surface and an external surface, wherein (i) at least one of the AAVs is not linked to the luminal surface of the EV or (ii) at least one of the AAVs is linked to the luminal surface of the EV.

7. The EV of claim 6, wherein the at least one AAV is linked to the luminal surface of the EV by a covalent bond, a non-covalent bond, or both.

8. The EV of any one of claims 1 to 7, wherein the AAV is linked to the scaffold protein.

9. The EV of any one of claims 1 to 8, wherein the scaffold protein comprises an N terminus domain (ND) and an effector domain (ED), wherein the ND and/or the ED are associated with the luminal surface of the EV,

wherein the ND is associated with the luminal surface of the EV via myristoylation, and

wherein the ED is associated with the luminal surface of the EV by an ionic interaction; and

wherein the ND comprises an amino acid sequence selected from the group consisting of (i) GGKLSKK (SEQ ID NO: 17), (ii) GAKLSKK (SEQ ID NO: 18), (iii) GGKQSKK (SEQ ID NO: 19), (iv) GGKLAKK (SEQ ID NO: 20), (v) GGKLSKK (SEQ ID NO: 21), or

(vi) GGKLSKKK (SEQ ID NO: 22), (vii) GGKLSKKS (SEQ ID NO: 23), (viii) GAKLSKKK (SEQ ID NO: 24), (ix) GAKLSKKS (SEQ ID NO: 25), (x) GGKQSKKK (SEQ ID NO: 26), (xi) GGKQSKKS (SEQ ID NO: 27), (xii) GGKLAKKK (SEQ ID NO: 28), (xiii) GGKLAKKS (SEQ ID NO: 29), (xiv) GGKLSKKK (SEQ ID NO: 30), (xv) GGKLSKKS (SEQ ID NO: 31), and (xvi) any combination thereof.

10. The EV of claim 9, wherein the scaffold protein comprises a myristoylated amino acid residue at the N terminus of the scaffold protein.

11. The EV of claim 10, wherein the amino acid residue at the N terminus of the scaffold protein is Gly.

12. The EV of claim 10 or 11, wherein the amino acid residue at the N terminus of the scaffold protein is synthetic or a glycine analog.

13. The EV of any one of claims 9 to 12, wherein the EV further comprises a second scaffold protein, which comprises prostaglandin F2 receptor negative regulator (the PTGFRN protein); basigin (the BSG protein); immunoglobulin superfamily member 2 (the IGSF2 protein); immunoglobulin superfamily member 3 (the IGSF3 protein); immunoglobulin superfamily member 8 (the IGSF8 protein); integrin beta-1 (the ITGB1 protein); integrin alpha-4 (the ITGA4 protein); 4F2 cell-surface antigen heavy chain (the SLC3A2 protein); a class of ATP transporter proteins (the ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B3, ATP2B1, ATP2B2, ATP2B3, ATP2B4 proteins), CD13, aminopeptidase N (ANPEP), neprilysin (membrane

metalloendopeptidase; MME), ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (ENPP1), neuropilin-1 (NRP1), CD9, CD63, CD81, PDGFR, GPI anchor proteins, lactadherin, LAMP2, LAMP2B, a fragment thereof, and any combination thereof.

14. The EV of any one of claims 1 to 13, wherein the AAV comprises at least one capsid protein fused to the scaffold protein and/or the second scaffold protein.

15. The EV of claim 14, wherein the AAV capsid protein comprises VP2.

16. The EV of claim 14 or 15, wherein the AAV comprises at least one VP2 that is not fused to the scaffold protein and/or the second scaffold protein.

17. The EV of any one of claims 14 to 16, wherein the scaffold protein is fused to the N-terminus of the VP2.

18. The EV of claim 16 or 17, wherein the number of the VP2 fused to the scaffold protein is less than the number of the at least one VP2 not fused to the scaffold protein.

19. The EV of any one of claims 1 to 8, wherein the scaffold protein is a type I transmembrane protein or a type II transmembrane protein.

20. The EV of claim 19, wherein the a type I transmembrane protein comprises prostaglandin F2 receptor negative regulator (the PTGFRN protein); basigin (the BSG protein); immunoglobulin superfamily member 2 (the IGSF2 protein); immunoglobulin superfamily member 3 (the IGSF3 protein); immunoglobulin superfamily member 8 (the IGSF8 protein); integrin beta-1 (the ITGB1 protein); integrin alpha-4 (the ITGA4 protein); 4F2 cell-surface antigen heavy chain (the SLC3A2 protein); a class of ATP transporter proteins (the ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B3, ATP2B1, ATP2B2, ATP2B3, ATP2B4 proteins), CD13, aminopeptidase N (ANPEP), neprilysin (membrane metalloendopeptidase; MME), ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (ENPP1), neuropilin-1 (NRP1), CD9, CD63, CD81, PDGFR, GPI anchor proteins, lactadherin, LAMP2, LAMP2B, a fragment thereof, and any combination thereof.

21. The EV of claim 19 or 20, wherein the C terminus of the type I transmembrane protein or the N terminus of the type II transmembrane protein is linked to a binding partner of a chemically induced dimer.

22. The EV of any one of claims 9 to 13, wherein the scaffold protein is linked to a binding partner of a chemically induced dimer.

23. The EV of claim 21 or 22, wherein the binding partner of the chemically induced dimer comprises one of binding partners selected from the group; consisting of (i) FKBP and FKBP (FK1012); (ii) FKBP and CalcineurinA (CNA) (FK506); (iii) FKBP and CyP-Fas (FKCsA); (iv) FKBP and FRB (Rapamycin); (v) GyrB and GyrB (Coumermycin); (vi) GAI and GID1 (Gibberellin); (vii) Snap-tag and HaloTag (HaXS); (viii) eDHFR and HaloTag (TMP-HTag); and (ix) BCL-xL and Fab (AZ1) (ABT-737).

24. An AAV in the EV of any one of claims 1 to 23.

25. An extracellular vesicle (EV) comprising (i) an adeno-associated virus (AAV) and (ii) a scaffold protein, wherein the AAV is associated with the scaffold protein on the external surface of the EV.

26. The EV of claim 25, wherein the scaffold protein comprises an extracellular domain, and wherein the AAV is associated with the extracellular domain of the scaffold protein.

27. The EV of claim 25 or 26, wherein the scaffold protein comprises a heterologous polypeptide, wherein the heterologous polypeptide is fused to an extracellular domain of the scaffold protein, and wherein the heterologous polypeptide associates with the AAV.

28. The EV of any one of claims 25 to 27, wherein the scaffold protein is a type I transmembrane protein or a type II transmembrane protein.

29. The EV of claim 27 or 28, wherein the heterologous polypeptide is fused to the N-terminus or the C terminus of the extracellular domain of the scaffold protein.

30. The EV of any one of claims 27 to 29, wherein the heterologous polypeptide comprises a receptor, a ligand, an antigen-binding moiety, a substrate, a fragment thereof, or a combination

thereof; and wherein the heterologous polypeptide specifically interacts with one or more proteins on the surface of the AAV.

31. The EV of claim 30, wherein the heterologous polypeptide comprises an antigen-binding moiety selected from the group consisting of an antigen-binding fragment of an antibody, a camelid antibody or an antigen-binding fragment thereof, a single-chain FAB, a nanobody, a shark IgNAR, and a combination thereof.

32. The EV of claim 30 or 31, wherein the antigen binding moiety specifically binds the one or more proteins on the surface of the AAV, wherein the one or more proteins on the surface of the AAV comprise (i) a capsid protein selected from the group consisting of VP1, VP2, VP3, and any combination thereof; or (ii) a non-AAV sequence fused to a capsid protein of the AAV.

33. The EV of claim 32, wherein the non-AAV sequence is fused to VP2.

34. The EV of claim 32 or 33, wherein the non-AAV sequence is fused to the N-terminus the capsid protein or to an internal surface-exposed loop of the capsid protein.

35. The EV of any one of claims 25 to 30, wherein:

(a) (i) the scaffold protein is fused to a heterologous polypeptide comprising an Fc receptor; and

(ii) the AAV comprises at least one capsid protein fused to an Fc region of an immunoglobulin constant region (Fc);

(b) (i) the scaffold protein is fused to a heterologous polypeptide comprising a nanobody; and

(ii) the AAV comprises at least one capsid protein fused to an Fc region of an immunoglobulin constant region (Fc);

(c) the scaffold protein is fused to a an antigen-binding moiety, wherein the antigen-binding moiety specifically binds an antigen on the surface of an AAV; or

(d) the scaffold protein is fused to a is fused to a heterologous polypeptide comprising an AAV receptor.

36. The EV of claim 34, wherein the surface-exposed loop comprises the sequence GTTTQSR (SEQ ID NO: 43) or amino acid residues 453 to 459 of VP1.

37. The EV of any one of claims 25 to 36, wherein the AAV further comprises a nucleotide sequence comprising a gene of interest.

38. The EV of claim 37, wherein the gene of interest encodes a protein selected from the group consisting of a secreted protein, a receptor, a structural protein, a signaling protein, a sensory protein, a regulatory protein, a transport protein, a storage protein, a defense protein, a motor protein, a clotting factor, a growth factor, an antioxidant, a cytokine, a chemokine, an enzyme, a tumor suppressor gene, a DNA repair protein, a structural protein, a low-density lipoprotein receptor, an alpha glucosidase, a cystic fibrosis transmembrane conductance regulator, or any combination thereof.

39. The EV of claim 37 or 38, wherein the gene of interest encodes a factor VIII protein, a Factor IX protein, or a Rab proteins geranylgeranyltransferase component A 1 (REP1)

40. The EV of any one of claims 25 to 39, wherein the AAV is selected from the group consisting of AAV type 1, AAV type 2, AAV type 3A, AAV type 3B, AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, snake AAV, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, a synthetic AAV, an any combination thereof.

41. The EV of any one of claims 25 to 40, wherein the scaffold protein is selected from the group consisting of prostaglandin F2 receptor negative regulator (the PTGFRN protein); basigin (the BSG protein); immunoglobulin superfamily member 2 (the IGSF2 protein); immunoglobulin superfamily member 3 (the IGSF3 protein); immunoglobulin superfamily member 8 (the IGSF8 protein); integrin beta-1 (the ITGB1 protein); integrin alpha-4 (the ITGA4 protein); 4F2 cell-surface antigen heavy chain (the SLC3A2 protein); a class of ATP transporter proteins (the ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B3, ATP2B1, ATP2B2, ATP2B3, ATP2B4 proteins), CD13, aminopeptidase N (ANPEP), neprilysin (membrane metalloendopeptidase;

MME), ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (ENPP1), neuropilin-1 (NRP1), CD9, CD63, CD81, PDGFR, GPI anchor proteins, lactadherin, LAMP2, LAMP2B, a fragment thereof, and any combination thereof.

42. The EV of any one of claims 25 to 41, wherein the scaffold protein is PTGFRN.

43. The EV of any one of claims 1 to 23 and 25 to 42, which is an exosome.

44. An adeno-associated virus (AAV) comprising a capsid, wherein the capsid comprises at least one capsid protein selected from the group consisting of VP1, VP2, and VP3; wherein the at least one capsid protein is linked to a scaffold protein.

45. A pharmaceutical composition comprising the EV of any one of claims 1 to 23, 25 to 43 or the AAV of claim 24 or 44 and a pharmaceutically acceptable carrier.

46. A cell that produces the isolated EV of any one of claims 1 to 23, 25 to 43 or the AAV of claim 24 or 44.

47. A kit comprising (i) the isolated EV of any one of claims 1 to 43 or the AAV of claim 24 or 44 and (ii) instructions for use.

48. Use of the EV of any one of claims 1 to 23, 25 to 43 or the AAV of claim 24 or 44 or the pharmaceutical composition of claim 45 in the manufacture of a medicament for the prevention or treatment of a disease in a subject in need thereof.

49. The use of claim 48, wherein the disease is selected from a cancer, a hemophilia, diabetes, a growth factor deficiency, an eye disease, a Pompe disease, a lysosomal storage disorder, mucovicidosis, cystic fibrosis, Duchenne and Becker muscular dystrophy, transthyretin amyloidosis, hemophilia A, hemophilia B, adenosine-deaminase deficiency, Leber's congenital amaurosis, X-linked adrenoleukodystrophy, metachromatic leukodystrophy, OTC deficiency, glycogen storage disease 1A, Criggler-Najjar syndrome, primary hyperoxaluria type 1, acute intermittent porphyria, phenylketonuria, familial hypercholesterolemia, mucopolysaccharidosis type VI, α1 antitrypsin deficiency, and a hypercholesterolemia.

50. The use of claim 48 or 49, wherein the EV is administered parenteral, oral, intravenous, intramuscular, intra-tumor, intranasal, subcutaneous, intraperitoneal, or intraocularly.

51. The use of any one of claims 48 to 50, wherein the subject is to be further administered an additional therapeutic agent.