

Claims:

1. A red blood cell extracellular vesicle (RBCEV) loaded with a DNA cargo.
2. The RBCEV of claim 1, wherein the DNA cargo is single stranded and has a length of at least 250
5 bases.
3. The RBCEV of claim 1 or 2, wherein the DNA cargo is single stranded and has a length of at least 2000 bases or 2000-30000 bases or more.
- 10 4. The RBCEV of claim 1, wherein the DNA cargo is double stranded and has a length of at least 250 base pairs.
5. The RBCEV of claim 1 or 4, wherein the DNA cargo is double stranded and has a length of at least 2000 base pairs or 2000-17000 base pairs or more.
- 15 6. The RBCEV of any preceding claim wherein the DNA cargo is an expression vector comprising a gene encoding a protein or peptide.
7. The RBCEV of any one of claims 1 to 6, wherein the DNA cargo is circular.
- 20 8. The RBCEV of any one of claims 1 to 7, wherein the DNA cargo is a minicircle or plasmid.
9. The RBCEV of any one of claims 1 to 6, wherein the DNA cargo is linear.
- 25 10. The RBCEV of any preceding claim wherein the DNA cargo is in the lumen of the RBCEV.
11. The RBCEV of any preceding claim wherein the RBCEV is derived or obtained from human or mammalian red blood cells.
- 30 12. The RBCEV of any preceding claim wherein the RBCEV is isolated.
13. An isolated red blood cell extracellular vesicle (RBCEV) containing in the lumen of the RBCEV at least one DNA cargo.
- 35 14. A composition comprising a plurality of RBCEVs according to any one of the preceding claims.
15. The composition of claim 14, wherein on average each RBCEV in the composition is loaded with at least 1.0 DNA cargo.
- 40 16. The composition of claim 14 or 15, wherein on average each RBCEV in the composition is loaded with at least 2.0 or 3.0 DNA cargoes.

17. The composition of any one of claims 14 to 16, wherein on average each RBCEV in the composition is loaded with 1.0 to 4.0 DNA cargoes.
- 5 18. The composition of any one of claims 14 to 17, wherein the composition is a pharmaceutical composition or medicament.
19. The composition of claim 18, wherein the composition or medicament further comprises a pharmaceutically acceptable carrier, diluent, excipient or stabiliser.
- 10 20. A method of treating a subject in need of treatment, the method comprising administering to the subject a therapeutically effective amount of an RBCEV according to any one of claims 1 to 13 or a composition according to any one of claims 14 to 19, thereby treating the subject.
- 15 21. The method of claim 20, wherein the method involves treatment of a disease in the subject by expression of a protein or peptide from a gene sequence of the DNA cargo.
22. The method of claim 21, wherein the treatment comprises prevention and/or amelioration of the disease.
- 20 23. A method for loading an extracellular vesicle with a nucleic acid cargo, the method comprising:
- a. providing a nucleic acid to be loaded into an extracellular vesicle;
 - b. contacting the nucleic acid with an extracellular vesicle in the presence of a transfection reagent under conditions sufficient for the extracellular vesicle to be loaded with the nucleic acid; and
 - 25 c. optionally washing the loaded extracellular vesicle.
24. The method of claim 23, wherein the extracellular vesicle is a red blood cell extracellular vesicle.
- 30 25. The method of claim 23 or 24, wherein the method comprises repeating step b.
26. A method for loading an extracellular vesicle with a nucleic acid cargo, the method comprising:
- a. providing a nucleic acid to be loaded into an extracellular vesicle;
 - b. contacting the nucleic acid with transfection reagent to allow formation of nucleic acid/transfection reagent complexes;
 - 35 c. incubating or contacting the nucleic acid/transfection reagent complexes with an extracellular vesicle under conditions sufficient for the extracellular vesicle to be loaded with a nucleic acid/transfection reagent complex; and
 - d. optionally washing the loaded extracellular vesicle.
- 40 27. A method for loading an extracellular vesicle with a nucleic acid cargo, the method comprising:
- a. providing a nucleic acid to be loaded into an extracellular vesicle;

- 5 b. contacting the nucleic acid with transfection reagent to allow formation of nucleic acid/transfection reagent complexes;
- c. incubating or contacting the nucleic acid/transfection reagent complexes with an extracellular vesicle under conditions sufficient for the extracellular vesicle to be loaded with a nucleic acid/transfection reagent complex;
- d. optionally washing the loaded extracellular vesicle;
- e. contacting the loaded extracellular vesicle with further nucleic acid/transfection reagent complexes; and
- 10 f. incubating or contacting the further nucleic acid/transfection reagent complexes with the loaded extracellular vesicle.
28. The method of claim 26 or 27, wherein the extracellular vesicle is a red blood cell extracellular vesicle.
- 15 29. The method of any one of claims 26 to 28, wherein the method further comprises repeating steps b-d.
30. The method of any one of claims 23 to 29, wherein the transfection reagent is a Linear Polyethylenimine Hydrochloride, optionally of MW 25,000Da or MW 40,000Da.
- 20 31. The method of any one of claims 23 to 29, wherein the method further comprises the step of removing nucleic acid cargo not contained within the lumen of the extracellular vesicle.
32. The method of claim 31, wherein removing nucleic acid cargo not contained within the lumen of the extracellular vesicle comprises contacting the loaded extracellular vesicle with a nuclease, preferably
25 an RNase or DNase.
33. The method of claim 32, wherein the loaded extracellular vesicle is contacted with heparin prior to contact with nuclease.
- 30 34. The method of any one of claims 23 to 33, wherein the nucleic acid cargo comprises nucleic acid molecules, wherein each nucleic acid molecule is single stranded and has a length of at least 250 bases, or at least 2000 bases, or 2000-30000 bases or more.
35. The method of any one of claims 23 to 33, wherein the nucleic acid cargo comprises nucleic acid
35 molecules, wherein each nucleic acid molecule is double stranded and has a length of at least 250 base pairs, or at least 2000 base pairs, or 2000-17000 base pairs or more.
36. The method of any one of claims 23 to 35, wherein the nucleic acid cargo is circular.
- 40 37. The method of claim 36, wherein the nucleic acid cargo is a minicircle or plasmid.
38. The method of any one of claims 23 to 35, wherein the nucleic acid cargo is linear.

39. The method of any one of claims 23 to 38, wherein the nucleic acid cargo is DNA.

40. The method of any one of claims 23 to 39, wherein the extracellular vesicle is a microvesicle.

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41. The method of any one of claims 23 to 39, wherein the extracellular vesicle is an exosome.

42. An extracellular vesicle loaded with a nucleic acid cargo, prepared or obtained by the method of any one of claims 23 to 41.

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