CLAIMS

1. A lentiviral vector comprising:

a lipid coat which comprises a CD47 polypeptide and does not comprise a MHC-I polypeptide; and

a nucleic acid comprising a nucleotide sequence encoding a polypeptide with FVIII activity, wherein the nucleotide sequence has:

(i) at least 91%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 1;

(ii) at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 2;

(iii) at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 70;

(iv) at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 71;

(v) at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 3;

(vi) at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 4;

(vii) at least 89%, 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%, or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 5; or

(viii) at least 89%, 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%, or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 6.

2. A lentiviral vector comprising:

a lipid coat which comprises a CD47 polypeptide and does not comprise a MHC-I polypeptide; and

a nucleic acid comprising a nucleotide sequence which comprises a first nucleic acid sequence encoding an N-terminal portion of a Factor VIII (FVIII) polypeptide and a second nucleic acid sequence encoding a C-terminal portion of a FVIII polypeptide;

wherein the first nucleic acid sequence comprises:

(i) at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotide[[s]] 58-2277 of SEQ ID NO: 3;

(ii) at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotide[[s]] 58-2277 of SEQ ID NO: 4;

(iii) at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotide[[s]] 58-2277 of SEQ ID NO: 5; or

(iv) at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotide[[s]] 58-2277 of SEQ ID NO: 6; and

wherein the second nucleic acid sequence comprises:

(i) at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotide 2320-4374 of SEQ ID NO: 3;

(ii) at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotide 2320-4374 of SEQ ID NO: 4;

(iii) at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotide 2320-4374 of SEQ ID NO: 5; or

(iv) at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotide 2320-4374 of SEQ ID NO: 6;

wherein the N-terminal portion and the C-terminal portion together have a FVIII polypeptide activity.

3. The lentiviral vector of claim 1 or claim 2, wherein the lentiviral vector is for use in a method of treating hemophilia A in a subject in need thereof that comprises administering to the subject in need thereof at least one dose ranging from about $1x10^8$ to about $5x10^{10}$ transducing units/kg (TU/kg) of the lentiviral vector.

4. The lentiviral vector of claim 3, wherein:

the dose is about $5x10^{10}$ TU/kg, about $4.5x10^{10}$ TU/kg, about $4x10^{10}$ TU/kg, about $3.5x10^{10}$ TU/kg, about $3x10^{10}$ TU/kg, about $2.5x10^{10}$ TU/kg, about $2x10^{10}$ TU/kg, about $1.5x10^{10}$ TU/kg, about $1x10^{10}$ TU/kg, about $9.5x10^9$ TU/kg, about $9x10^9$ TU/kg, about $8.5x10^9$ TU/kg, about $8x10^9$ TU/kg, about $7.5x10^9$ TU/kg, about $7x10^9$ TU/kg, about $6.5x10^9$ TU/kg,

about $6x10^9$ TU/kg, about $5.5x10^9$ TU/kg, about $5x10^9$ TU/kg, about $4.5x10^9$ TU/kg, about $4x10^9$ TU/kg, about $3.5x10^9$ TU/kg, about $3x10^9$ TU/kg, about $2.5x10^9$ TU/kg, about $2x10^9$ TU/kg, about $1.5x10^9$ TU/kg, about $1x10^9$ TU/kg, about $9.5x10^8$ TU/kg, about $9x10^8$ TU/kg, about $8.5x10^8$ TU/kg, about $8x10^8$ TU/kg, about $7.5x10^8$ TU/kg, about $7x10^8$ TU/kg, about $6.5x10^8$ TU/kg, about $6x10^8$ TU/kg, about $5.5x10^8$ TU/kg, about $5x10^8$ TU/kg, about $4.5x10^8$ TU/kg, about $4x10^8$ TU/kg, about $3.5x10^8$ TU/kg, about $3x10^8$ TU/kg, about $2.5x10^8$ TU/kg, about $2x10^8$ TU/kg, about $1.5x10^8$ TU/kg, or about $1x10^8$ TU/kg;

the dose is between $1x10^8$ and $5x10^9$ TU/kg, between $1x10^8$ and $1x10^9$ TU/kg, between $1x10^8$ and $1x10^{10}$ TU/kg, between $1x10^9$ and $5x10^{10}$ TU/kg, between $2x10^9$ and $5x10^{10}$ TU/kg, between $3x10^9$ and $5x10^{10}$ TU/kg, between $4x10^9$ and $5x10^{10}$ TU/kg, between $5x10^9$ and $5x10^{10}$ TU/kg, between $6x10^9$ and $5x10^{10}$ TU/kg, between $7x10^9$ and $5x10^{10}$ TU/kg, $8x10^9$ and $5x10^{10}$ TU/kg, between $9x10^9$ and $5x10^{10}$ TU/kg, between 10^{10} and $5x10^{10}$ TU/kg, between $1.5x10^{10}$ and $5x10^{10}$ TU/kg, between $2x10^{10}$ and $5x10^{10}$ TU/kg, between $2.5x10^{10}$ and $5x10^{10}$ TU/kg, between $3x10^{10}$ and $5x10^{10}$ TU/kg, between $3.5x10^{10}$ and $5x10^{10}$ TU/kg, between $4x10^{10}$ and $5x10^{10}$ TU/kg, or between $4.5x10^{10}$ and $5x10^{10}$ TU/kg;

the dose is between $1x10^9$ and $5x10^{10}$ TU/kg, between $1x10^9$ and $4.5x10^{10}$ TU/kg, between $1x10^9$ and $4x10^{10}$ TU/kg, between $1x10^9$ and $3.5x10^{10}$ TU/kg, between $1x10^9$ and $3x10^{10}$ TU/kg, between $1x10^9$ and $2.5x10^{10}$ TU/kg, between $1x10^9$ and $2x10^{10}$ TU/kg, between $1x10^9$ and $1.5x10^{10}$ TU/kg, between $1x10^9$ and $1x10^{10}$ TU/kg, between $1x10^9$ and $9x10^9$ TU/kg, between $1x10^9$ and $8x10^9$ TU/kg, between $1x10^9$ and $7x10^9$ TU/kg, between $1x10^9$ and $6x10^9$ TU/kg, between $1x10^9$ and $5x10^9$ TU/kg, between $1x10^9$ and $4x10^9$ TU/kg, between $1x10^9$ and $3x10^9$ TU/kg, and between $1x10^9$ and $2x10^9$ TU/kg;

the dose is between $1x10^{10}$ and $2x10^{10}$ TU/kg, between $1.1x10^{10}$ and $1.9x10^{10}$ TU/kg, between $1.2x10^{10}$ and $1.8x10^{10}$ TU/kg, between $1.3x10^{10}$ and $1.7x10^{10}$ TU/kg, or between $1.4x10^{10}$ and $1.6x10^{10}$ TU/kg;

the dose is between $2.5x10^9$ TU/kg and $3.5x10^9$ TU/kg, between $2.6\ x10^9$ TU/kg and $3.4x10^9$ TU/kg, between $2.7x10^9$ TU/kg and $3.3x10^9$ TU/kg, between $2.8x10^9$ TU/kg and $3.2x10^9$ TU/kg, or between $2.9x10^9$ TU/kg and $3.1x10^9$ TU/kg; or

the dose is between $5.5x10^9$ TU/kg and $6.5x10^9$ TU/kg, between $5.6\ x10^9$ TU/kg and $6.4x10^9$ TU/kg, between $5.7x10^9$ TU/kg and $6.3x10^9$ TU/kg, between $5.8x10^9$ TU/kg and $6.2x10^9$ TU/kg, or between $5.9x10^9$ TU/kg and $6.1x10^9$ TU/kg.

5. The lentiviral vector of claim 3, wherein the dose is about $1.5x10^{10}$ TU/kg, about $1.0x10^9$ TU/kg, about $3.0x10^9$ TU/kg, about $6.0x10^9$ TU/kg, about $1x10^8$ TU/kg, about $8.3x10^8$ TU/kg, about $1.5x10^9$ TU/kg, about $4.5x10^9$ TU/kg, or about $1.3x10^{10}$ TU/kg.

6. The lentiviral vector of any one of claims 3-5, wherein when the lentiviral vector is administered to a subject, plasma FVIII activity in the subject is increased at 24 hours to 48 hours post administration of the lentiviral vector-relative to a subject administered a reference vector comprising a nucleic acid comprising SEQ ID NO: 16; and

optionally wherein the plasma FVIII activity is increased by at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 11-fold, at least about 12-fold, at least about 13-fold, at least about 14-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 50-fold, at least about 60-fold, at least about 70-fold, at least about 80-fold, at least about 90-fold, at least about 100-fold, at least about 110-fold, at least about 120-fold, at least about 130-fold, at least about 140-fold, at least about 150-fold, at least about 160-fold, at least about 170-fold, at least about 180-fold, at least about 190-fold, or at least about 200-fold.

7. The lentiviral vector of any one of claims 3 to 6, wherein the lentiviral vector is formulated to be administered to the subject as a single dose or multiple doses.

8. The lentiviral vector of any one of claims 3 to 7, wherein the lentiviral vector is formulated to be administered to the subject via intravenous injection.

9. The lentiviral vector of any one of claims 3 to 8, wherein the subject is an adult subject.

10. The lentiviral vector of any one of claims 1 to 9, wherein the lentiviral vector comprises a tissue specific promoter, optionally wherein the tissue specific promoter selectively enhances expression of the polypeptide with FVIII activity in a target liver cell; and/or wherein the tissue specific promoter selectively enhances expression of the polypeptide with FVIII activity in a target liver cell comprises an mTTR promoter.

11. The lentiviral vector of claim 10, wherein the target liver cell is a hepatocyte, and optionally wherein the nucleic acid is stably integrated into the genome of the hepatocyte.

12. The lentiviral vector of any one of claims 1 to 11, wherein nucleotide sequence encoding a polypeptide with FVIII activitycomprises LV-coFVIII-6 (SEQ ID NO:71).

13. The lentiviral vector of any one of claims 1 to 11, wherein the nucleotide sequence encoding a polypeptide with FVIII activity comprises LV-coFVIII-6-XTEN (SEQ ID NO:72).

14. The method of any one of claims 3 to 13, wherein the dose of lentivirus vector is administered to the subject once or divided into at least two sub-doses.

15. The lentiviral vector of any one of claims 1 to 14, wherein the nucleotide sequence encoding a polypeptide with FVIII activity further comprises a nucleic acid sequence encoding a signal peptide, wherein the nucleic acid sequence encoding a signal peptide has at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to:

(i) nucleotides 1 to 57 of SEQ ID NO: 1;
(ii) nucleotides 1 to 57 of SEQ ID NO: 2;
(iii) nucleotides 1 to 57 of SEQ ID NO: 3;
(iv) nucleotides 1 to 57 of SEQ ID NO: 4;
(v) nucleotides 1 to 57 of SEQ ID NO: 5;
(vi) nucleotides 1 to 57 of SEQ ID NO: 6;
(vii) nucleotides 1 to 57 of SEQ ID NO: 70;
(viii) nucleotides 1 to 57 of SEQ ID NO: 71; or
(ix) nucleotides 1 to 57 of SEQ ID NO: 68.

16. The lentiviral vector of any one of claims 1 to 15, wherein the nucleotide sequence encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide comprises one or more properties selected from the group consisting of:

(a) the human codon adaptation index of the nucleotide sequence encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide is increased relative to SEQ ID NO: 16;

(b) the frequency of optimal codons of the nucleotide sequence encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide is increased relative to SEQ ID NO:16;

(c) the nucleotide sequence encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII

polypeptide contains a higher percentage of G/C nucleotides compared to the percentage of G/C nucleotides in SEQ ID NO: 16;

(d) the relative synonymous codon usage of the nucleotide sequence encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide is increased relative to SEQ ID NO: 16;

(e) the effective number of codons of the nucleotide sequence encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide is reduced relative SEQ ID NO: 16;

(f) the nucleotide sequence encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide contains fewer MARS/ARS sequences (SEQ ID NOs: 21 and 22) relative to SEQ ID NO: 16;

(g) the nucleotide sequence encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide contains fewer destabilizing elements (SEQ ID NOs: 23 and 24) relative to SEQ ID NO: 16; and

(h) any combination thereof.

17. The lentiviral vector of any one of claims 1 to 16, wherein the nucleotide sequence encoding a polypeptide with FVIII activity further comprises a heterologous nucleotide sequence encoding a heterologous amino acid sequence, optionally wherein the heterologous amino acid sequence is an immunoglobulin constant region or a portion thereof, XTEN, transferrin, albumin, or a PAS sequence, and more preferably wherein the heterologous amino acid sequence is linked to the N-terminus or the C-terminus of the amino acid sequence encoded by a nucleotide sequence encoding a polypeptide with FVIII activity or inserted between two amino acids in the amino acid sequence encoded by the nucleotide sequence at one or more insertion sites selected from TABLE 3.

18. The lentiviral vector of any one of claims 1 to 17, wherein the FVIII polypeptide is a full length FVIII or a B domain deleted FVIII.

19. The lentiviral vector of any one of claims 1 to 18, wherein the CD47 polypeptide is a human CD47 polypeptide.

20. The lentiviral vector of any one of claims 1 to 19, wherein the lentiviral vector is produced in a host cell, optionally wherein:

the host cell expresses CD47;

the host cell does not express MHC-I;

the host cell is $CD47^{high}$/MHC-I^{-}; or

the host cell is a $CD47^{high}$/MHC-I^{-} HEK 293T cell.

21. The lentiviral vector of claim 20, wherein the lentiviral vector is produced in a CD47high/MHC-I- host cell.

22. The lentiviral vector of claim 21, wherein the host cell is a HEK 293T cell modified to have increased expression of CD47 relative to unmodified HEK 293T cells.