We claim:

1. A composition comprising a nucleic acid construct comprising a first factor IX protein coding sequence or a reverse complement of the first factor IX protein coding sequence.

2. The composition of claim 1, wherein the first factor IX protein coding sequence:

(I) is at least 95% or at least 99% identical to SEQ ID NO: 166, is CpG-depleted, and is codon-optimized, and optionally wherein the factor IX protein is not a hyperactive factor IX variant, optionally wherein the first factor IX protein coding sequence has all but one CpG dinucleotides removed or is fully CpG depleted; or

(II) is at least 99% identical to SEQ ID NO: 159, is CpG-depleted, and is modified to mutate one or more cryptic splice donor sequences, and optionally wherein the factor IX protein is not a hyperactive factor IX variant, optionally wherein the first factor IX protein coding sequence has all but one CpG dinucleotides removed or is fully CpG depleted.

3. The composition of any preceding claim, wherein the first factor IX protein coding sequence encodes a factor IX protein comprising SEQ ID NO: 195.

4. The composition of claim 1, wherein the first factor IX protein coding sequence comprises SEQ ID NO: 166 or the first factor IX protein coding sequence consists of SEQ ID NO: 166.

5. The composition of claim 1, wherein the first factor IX protein coding sequence comprises SEQ ID NO: 159 or the first factor IX protein coding sequence consists of SEQ ID NO: 159.

6. The composition of any preceding claim, wherein the nucleic acid construct comprises a splice acceptor upstream of the first factor IX protein coding sequence, and the nucleic acid construct comprises a polyadenylation signal downstream of the first factor IX protein coding sequence.

7. The composition of any preceding claim, wherein the nucleic acid construct does not comprise homology arms, and wherein the nucleic acid construct does not comprise a promoter that drives the expression of the factor IX protein.

8. The composition of any preceding claim, wherein the nucleic acid construct is a bidirectional construct, wherein the nucleic acid construct comprises the first factor IX protein coding sequence and a reverse complement of a second factor IX protein coding sequence, or wherein the nucleic acid construct comprises the second factor IX protein coding sequence and the reverse complement of the first factor IX protein coding sequence,

wherein the first factor IX protein coding sequence and the second factor IX protein coding sequence are different but encode the same factor IX protein sequence, and optionally wherein the factor IX protein is not a hyperactive factor IX variant.

9. The composition of claim 8, wherein:

(I) the first factor IX protein coding sequence is at least 99% identical to SEQ ID NO: 159 and is: (i) CpG-depleted; (ii) modified to mutate one or more cryptic splice donor sequences; or (iii) CpG-depleted and modified to mutate one or more cryptic splice donor sequences, and optionally wherein the factor IX protein is not a hyperactive factor IX variant, optionally wherein the first factor IX protein coding sequence has all but one CpG dinucleotides removed or is fully CpG depleted; and

(II) the second factor IX protein coding sequence is at least 95% or at least 99% identical to SEQ ID NO: 166, is CpG-depleted, and is codon-optimized, and optionally wherein the factor IX protein is not a hyperactive factor IX variant, optionally wherein the second factor IX protein coding sequence has all but one CpG dinucleotides removed or is fully CpG depleted.

10. The composition of claim 8 or 9, wherein the first factor IX protein coding sequence encodes a factor IX protein comprising SEQ ID NO: 195, and the second factor IX protein coding sequence encodes a factor IX protein comprising SEQ ID NO: 195.

11. The composition of any one of claims 8-10, wherein:

(I) the first factor IX protein coding sequence comprises SEQ ID NO: 159 or the first factor IX protein coding sequence consists of SEQ ID NO: 159; and

(II) the second factor IX protein coding sequence comprises SEQ ID NO: 166 or the second factor IX protein coding sequence consists of SEQ ID NO: 166.

12. The composition of any one of claim 8-11, wherein the nucleic acid construct comprises from 5' to 3': a first splice acceptor, the first factor IX protein coding sequence, a first polyadenylation signal, a reverse complement of a second polyadenylation signal, the reverse complement of the second factor IX protein coding sequence, and a reverse complement of a second splice acceptor, or wherein the nucleic acid construct comprises from 5' to 3': a first splice acceptor, the second factor IX protein coding sequence, a first polyadenylation signal, a reverse complement of a second polyadenylation signal, the reverse complement of the first factor IX protein coding sequence, and a reverse complement of a second splice acceptor,

wherein the first factor IX protein coding sequence and the second factor IX protein coding sequence are different but encode the same factor IX protein sequence, and wherein the first polyadenylation signal and the second polyadenylation signal are different.

13. The composition of any preceding claim, wherein the nucleic acid construct is a bidirectional construct comprising the first factor IX protein coding sequence and a reverse complement of a second factor IX protein coding sequence,

wherein the nucleic acid construct comprises from 5' to 3': a first splice acceptor, the first factor IX protein coding sequence, a first polyadenylation signal, a reverse complement of a second polyadenylation signal, the reverse complement of the second factor IX protein coding sequence, and a reverse complement of a second splice acceptor,

wherein: (i) the first factor IX protein coding sequence comprises SEQ ID NO: 159 and the second factor IX protein coding sequence comprises SEQ ID NO: 166; or (ii) the first factor IX protein coding sequence comprises SEQ ID NO: 166 and the second factor IX protein coding sequence comprises SEQ ID NO: 159,

wherein the nucleic acid construct does not comprise a promoter that drives the expression of the factor IX protein, and

wherein the nucleic acid construct does not comprise homology arms.

14. The composition of any preceding claim, wherein the nucleic acid construct comprises SEQ ID NO: 210 or 180 or the reverse complement thereof.

15. The composition of any preceding claim, wherein the nucleic acid construct is in a nucleic acid vector, wherein the nucleic acid vector is an adeno-associated viral (AAV)vector,

optionally wherein the nucleic acid construct is flanked by inverted terminal repeats (ITRs) on each end, optionally wherein the ITR on at least one end comprises, consists essentially of, or consists of SEQ ID NO: 198, and optionally wherein the ITR on each end comprises, consists essentially of, or consists of SEQ ID NO: 198, or optionally wherein the ITR on at least one end comprises, consists essentially of, or consists of SEQ ID NO: 196, and optionally wherein the ITR on each end comprises, consists essentially of, or consists of SEQ ID NO: 196,

optionally wherein the AAV vector is a single-stranded AAV (ssAAV) vector,

optionally wherein the AAV vector is a recombinant AAV8 (rAAV8) vector, and

optionally wherein the AAV vector is a single-stranded rAAV8 vector.

16. The composition of any preceding claim, wherein the nucleic acid construct is a bidirectional construct comprising the first factor IX protein coding sequence and a reverse complement of a second factor IX protein coding sequence,

wherein the nucleic acid construct comprises from 5’ to 3’: a first splice acceptor, the first factor IX protein coding sequence, a first polyadenylation signal, a reverse complement of a second polyadenylation signal, the reverse complement of the second factor IX protein coding sequence, and a reverse complement of a second splice acceptor,

wherein: (i) the first factor IX protein coding sequence comprises SEQ ID NO: 159 and the second factor IX protein coding sequence comprises SEQ ID NO: 166; or (ii) the first factor IX protein coding sequence comprises SEQ ID NO: 166 and the second factor IX protein coding sequence comprises SEQ ID NO: 159,

wherein the nucleic acid construct does not comprise a promoter that drives the expression of the factor IX protein,

wherein the nucleic acid construct does not comprise homology arms, and

wherein the nucleic acid construct is in a single-stranded rAAV8 vector,

optionally wherein the nucleic acid construct is flanked by inverted terminal repeats (ITRs) on each end, optionally wherein the ITR on at least one end comprises, consists essentially of, or consists of SEQ ID NO: 198, and optionally wherein the ITR on each end comprises, consists essentially of, or consists of SEQ ID NO: 198, or optionally wherein the ITR on at least one end comprises, consists essentially of, or consists of SEQ ID NO: 196, and optionally wherein the ITR on each end comprises, consists essentially of, or consists of SEQ ID NO: 196.

17. The composition of any one of claims 1-7, wherein the nucleic acid construct is a unidirectional construct.

18. The composition of any preceding claim, further comprising a nuclease agent that targets a nuclease target site in a target gene, optionally wherein the target gene is an *albumin* gene, optionally wherein the *albumin* gene is a human *albumin* gen.

19. The composition of claim 18, wherein the nuclease agent comprises:

(a) a zinc finger nuclease (ZFN);

(b) a transcription activator-like effector nuclease (TALEN); or

(c) (i) a Cas protein or a nucleic acid encoding the Cas protein; and

(ii) a guide RNA or one or more DNAs encoding the guide RNA, wherein the guide RNA comprises a DNA-targeting segment that targets a guide RNA target sequence, and wherein the guide RNA binds to the Cas protein and targets the Cas protein to the guide RNA target sequence.

20. The composition of claim 18, wherein the nuclease agent comprises:

(a) a Cas protein or a nucleic acid encoding the Cas protein; and

(b) a guide RNA or one or more DNAs encoding the guide RNA, wherein the guide RNA comprises a DNA-targeting segment that targets a guide RNA target sequence in intron 1 of a human *albumin* gene, and wherein the guide RNA binds to the Cas protein and targets the Cas protein to the guide RNA target sequence.

21. The composition of claim 20, wherein the DNA-targeting segment comprises any one of SEQ ID NOS: 36, 30-35, and 37-61, optionally wherein the DNA-targeting segment comprises any one of SEQ ID NOS: 36, 30, 33, and 41, or

wherein the DNA-targeting segment consists of any one of SEQ ID NOS: 36, 30-35, and 37-61, optionally wherein the DNA-targeting segment consists of any one of SEQ ID NOS: 36, 30, 33, and 41, or

wherein the guide RNA comprises any one of SEQ ID NOS: 68, 100, 62-67, 69-99, and 101-125, optionally wherein the guide RNA comprises any one of SEQ ID NOS: 68, 100, 62, 94, 65, 97, 73, and 105.

22. The composition of claim 20 or 21, wherein the DNA-targeting segment comprises SEQ ID NO: 36, or wherein the DNA-targeting segment consists of SEQ ID NO: 36.

23. The composition of any one of claims 20-22, wherein the guide RNA comprises SEQ ID NO: 68 or 100, wherein the composition comprises the guide RNA in the form of RNA.

24. The composition of any one of claims 20-23, wherein the composition comprises the guide RNA in the form of RNA, the guide RNA comprises SEQ ID NO: 100, and the guide RNA comprises: (i) phosphorothioate bonds between the first four nucleotides at the 5' end of the guide RNA; (ii) phosphorothioate bonds between the last four nucleotides at the 3' end of the guide RNA; (iii) 2'-O-methyl-modified nucleotides at the first three nucleotides at the 5' end of the guide RNA; and (iv) 2'-O-methyl-modified nucleotides at the last three nucleotides at the 3' end of the guide RNA.

25. The composition of any one of claims 20-24, wherein the Cas protein is a Cas9 protein, optionally wherein the Cas protein is derived from a *Streptococcus pyogenes* Cas9 protein.

26. The composition of any one of claims 20-25, wherein the Cas protein comprises the sequence set forth in SEQ ID NO: 11.

27. The composition of any one of claims 20-26, wherein the composition comprises the nucleic acid encoding the Cas protein, wherein the nucleic acid comprises an

mRNA encoding the Cas protein, wherein the mRNA encoding the Cas protein is fully substituted with N1-methyl-pseudouridine.

28. The composition of claim 27, wherein the mRNA encoding the Cas protein comprises the sequence set forth in SEQ ID NO: 226 or 225.

29. The composition of any one of claims 20-28, wherein the composition comprises the nucleic acid encoding the Cas protein, wherein the nucleic acid comprises an mRNA encoding the Cas protein, the mRNA encoding the Cas protein comprises the sequence set forth in SEQ ID NO: 226 or 225, and the mRNA encoding the Cas protein is fully substituted with N1-methyl-pseudouridine, comprises a 5' cap, and comprises a poly(A) tail.

30. The composition of any one of claims 20-28, wherein the composition comprises the guide RNA in the form of RNA, and the guide RNA comprises SEQ ID NO: 68 or 100, and

wherein the composition comprises the nucleic acid encoding the Cas protein, wherein the nucleic acid comprises an mRNA encoding the Cas protein, and the mRNA encoding the Cas protein comprises the sequence set forth in SEQ ID NO: 226 or 225.

31. The composition of any one of claims 20-28, wherein the composition comprises the guide RNA in the form of RNA, the guide RNA comprises SEQ ID NO: 100, and the guide RNA comprises: (i) phosphorothioate bonds between the first four nucleotides at the 5' end of the guide RNA; (ii) phosphorothioate bonds between the last four nucleotides at the 3' end of the guide RNA; (iii) 2'-O-methyl-modified nucleotides at the first three nucleotides at the 5' end of the guide RNA; and (iv) 2'-O-methyl-modified nucleotides at the last three nucleotides at the 3' end of the guide RNA, and

wherein the composition comprises the nucleic acid encoding the Cas protein, wherein the nucleic acid comprises an mRNA encoding the Cas protein, the mRNA encoding the Cas protein comprises the sequence set forth in SEQ ID NO: 226 or 225, and the mRNA encoding the Cas protein is fully substituted with pseudouridine or N1-methyl-pseudouridine, optionally N1-methyl-pseudouridine, comprises a 5' cap, and comprises a poly(A) tail.

32. The composition of claim 30 or 31, wherein the nucleic acid construct is a bidirectional construct comprising the first factor IX protein coding sequence and a reverse complement of a second factor IX protein coding sequence,

wherein the nucleic acid construct comprises from 5’ to 3’: a first splice acceptor, the first factor IX protein coding sequence, a first polyadenylation signal, a reverse complement of a second polyadenylation signal, the reverse complement of the second factor IX protein coding sequence, and a reverse complement of a second splice acceptor,

wherein: (i) the first factor IX protein coding sequence comprises SEQ ID NO: 159 and the second factor IX protein coding sequence comprises SEQ ID NO: 166; or (ii) the first factor IX protein coding sequence comprises SEQ ID NO: 166 and the second factor IX protein coding sequence comprises SEQ ID NO: 159,

wherein the nucleic acid construct does not comprise a promoter that drives the expression of the factor IX protein, and

wherein the nucleic acid construct does not comprise homology arms.

33. The composition of any one of claims 20-32, wherein the Cas protein or the nucleic acid encoding the Cas protein and the guide RNA or the one or more DNAs encoding the guide RNA are associated with a lipid nanoparticle,

optionally wherein the lipid nanoparticle comprises a cationic lipid, a neutral lipid, a helper lipid, and a stealth lipid,

optionally wherein the cationic lipid is Lipid A, the neutral lipid is DSPC, the helper lipid is cholesterol, and the stealth lipid is PEG2k-DMG, and

optionally wherein the lipid nanoparticle comprises four lipids at the following molar ratios: about 50 mol% Lipid A, about 9 mol% DSPC, about 38 mol% cholesterol, and about 3 mol% PEG2k-DMG.

34. The composition of any one of claims 20-33, wherein the *albumin* gene is a human *albumin* gene,

wherein the composition comprises the guide RNA in the form of RNA, and the guide RNA comprises SEQ ID NO: 68 or 100,

wherein the composition comprises the nucleic acid encoding the Cas protein, wherein the nucleic acid comprises an mRNA encoding the Cas protein, and the mRNA encoding the Cas protein comprises the sequence set forth in SEQ ID NO: 226 or 225, and

wherein the guide RNA and the mRNA encoding the Cas protein are associated with a lipid nanoparticle comprising Lipid A, DSPC, cholesterol, and PEG2k-DMG, optionally at the following molar ratios: about 50 mol% Lipid A, about 9 mol% DSPC, about 38 mol% cholesterol, and about 3 mol% PEG2k-DMG.

35. The composition of any one of claims 20-33, wherein the *albumin* gene is a human *albumin* gene,

wherein the composition comprises the guide RNA in the form of RNA, the guide RNA comprises SEQ ID NO: 100, and the guide RNA comprises: (i) phosphorothioate bonds between the first four nucleotides at the 5' end of the guide RNA; (ii) phosphorothioate bonds between the last four nucleotides at the 3' end of the guide RNA; (iii) 2'-O-methyl-modified nucleotides at the first three nucleotides at the 5' end of the guide RNA; and (iv) 2'-O-methyl-modified nucleotides at the last three nucleotides at the 3' end of the guide RNA,

wherein the composition comprises the nucleic acid encoding the Cas protein, wherein the nucleic acid comprises an mRNA encoding the Cas protein, the mRNA encoding the Cas protein comprises the sequence set forth in SEQ ID NO: 226 or 225, and the mRNA encoding the Cas protein is fully substituted with pseudouridine or N1-methyl-pseudouridine, optionally N1-methyl-pseudouridine, comprises a 5' cap, and comprises a poly(A) tail, and

wherein the guide RNA and the mRNA encoding the Cas protein are associated with a lipid nanoparticle comprising Lipid A, DSPC, cholesterol, and PEG2k-DMG, optionally at the following molar ratios: about 50 mol% Lipid A, about 9 mol% DSPC, about 38 mol% cholesterol, and about 3 mol% PEG2k-DMG.

36. The composition of claim 34 or 35, wherein the nucleic acid construct is a bidirectional construct comprising the first factor IX protein coding sequence and a reverse complement of a second factor IX protein coding sequence,

wherein the nucleic acid construct comprises from 5' to 3': a first splice acceptor, the first factor IX protein coding sequence, a first polyadenylation signal, a reverse complement

of a second polyadenylation signal, the reverse complement of the second factor IX protein coding sequence, and a reverse complement of a second splice acceptor,

wherein: (i) the first factor IX protein coding sequence comprises SEQ ID NO: 159 and the second factor IX protein coding sequence comprises SEQ ID NO: 166; or (ii) the first factor IX protein coding sequence comprises SEQ ID NO: 166 and the second factor IX protein coding sequence comprises SEQ ID NO: 159,

wherein the nucleic acid construct does not comprise a promoter that drives the expression of the factor IX protein,

wherein the nucleic acid construct does not comprise homology arms, and

wherein the nucleic acid construct is in a single-stranded rAAV8 vector,

optionally wherein the nucleic acid construct is flanked by inverted terminal repeats (ITRs) on each end, optionally wherein the ITR on at least one end comprises, consists essentially of, or consists of SEQ ID NO: 198, and optionally wherein the ITR on each end comprises, consists essentially of, or consists of SEQ ID NO: 198, or optionally wherein the ITR on at least one end comprises, consists essentially of, or consists of SEQ ID NO: 196, and optionally wherein the ITR on each end comprises, consists essentially of, or consists of SEQ ID NO: 196.

37. A cell comprising the composition of any one of claims 1-36.

38. The cell of claim 37, wherein the nucleic acid construct is integrated into an endogenous target gene locus, and wherein factor IX protein is expressed from the endogenous target gene locus, or wherein the nucleic acid construct is integrated into intron 1 of an endogenous *albumin* locus, and wherein factor IX protein is expressed from the endogenous *albumin* locus.

39. The cell of claim 37 or 38, wherein:

(I) the cell is a human cell; and/or

(II) the cell is a liver cell or a hepatocyte; and/or

(III) the cell is not a neonatal cell or the cell is a neonatal cell, wherein the neonatal cell is from a human neonatal subject within 24 weeks after birth, within 12 weeks after birth, within 8 weeks after birth, or within 4 weeks after birth; and/or

(IV) the cell is *ex vivo* or *in vitro*.

40. The composition of any one of claims 1-36, for use in introducing a *factor 9* nucleic acid into a cell.

41. The composition of any one of claims 18-36, for use in integrating a *factor 9* nucleic acid construct into a target gene in a cell,

wherein the nuclease agent cleaves the nuclease target site in the target gene to create a cleavage site, the nucleic acid construct is inserted into the cleavage site to create a modified target gene, and factor IX protein is expressed from the modified target gene.

42. The composition of any one of claims 18-36, for use in expressing factor IX in a cell,

wherein the nuclease agent cleaves the nuclease target site in the target gene to create a cleavage site, the nucleic acid construct is inserted into the cleavage site to create a modified target gene, and factor IX protein is expressed from the modified target gene.

43. The composition for use according to any one of claims 40-42, wherein:

(I) the cell is a liver cell or a hepatocyte; and/or

(II) the cell is a human cell; and/or

(III) the cell is not a neonatal cell or the cell is a neonatal cell, wherein the neonatal cell is from a human neonatal subject within 24 weeks after birth, within 12 weeks after birth, within 8 weeks after birth, or within 4 weeks after birth; and/or

(IV) the cell is *in vivo*.

44. The composition of any one of claims 18-36, for use in treating a factor IX deficiency in a subject.

45. The composition of any one of claims 18-36, for use in treating hemophilia B in a subject.

46. The composition of any one of claims 18-36, for use in preventing or inhibiting spontaneous bleeding in a subject having hemophilia B, optionally wherein the hemophilia B is mild hemophilia B, moderate hemophilia B, or severe hemophilia B.

47. The composition for use according to any one of claims 43-46, wherein:

(I) the subject is not a neonatal subject, or the subject is a neonatal subject, wherein the neonatal subject is a human neonatal subject within 24 weeks after birth, within 12 weeks after birth, within 8 weeks after birth, or within 4 weeks after birth; and/or

(II) the subject is a human subject.

48. The composition for use according to any one of claims 43-47, wherein:

(I) the use results in a therapeutically effective level of circulating factor IX protein in the subject or a therapeutically effective level of circulating factor IX coagulation activity in the subject; and/or

(II) the use results in increased expression of the factor IX protein in the subject compared to use of an episomal expression vector encoding the factor IX protein in treating a control subject or wherein the use results in increased serum levels of the factor IX protein in the subject compared to use of an episomal expression vector encoding the factor IX protein in treating a control subject; and/or

(III) the use results in serum levels of the factor IX protein in the subject of at least about 1 μg/mL, at least about 2 μg/mL, at least about 3 μg/mL, at least about 4 μg/mL, or at least about 5 μg/mL, optionally wherein the use results in serum levels of the factor IX protein in the subject of at least about 5 μg/mL, or optionally wherein the use results in serum levels of the factor IX protein in the subject of between about 1 μg/mL and about 10 μg/mL; and/or

(IV) the use achieves circulatory factor IX protein or coagulation activity levels of at least about 1% of normal, at least about 5% of normal, at least about 10% of normal, at least about 15% of normal, at least about 20% of normal, at least about 25% of normal, at least about 30% of normal, at least about 35% of normal, at least about 40% of normal, at least about 45% of normal, or at least about 50% of normal, optionally wherein the use achieves circulatory factor IX protein or coagulation activity levels of between about 40% and about 150% or between about 40% and about 100% of normal, optionally wherein the use achieves circulatory factor IX protein or coagulation activity levels of at least about 15% of normal; and/or

(V) (a) the subject has severe hemophilia, and the use achieves circulatory factor IX protein or coagulation activity levels of at least about 1% or more than about 1% of normal;

(b) the subject has moderate hemophilia, and the use achieves circulatory factor IX protein or coagulation activity levels of at least about 5% or more than about 5% of normal; or

(c) the subject has mild hemophilia, and the use achieves circulatory factor IX protein or coagulation activity levels of at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, more than about 10%, more than about 15%, more than about 20%, more than about 25%, more than about 30%, more than about 35%, more than about 40%, more than about 45%, or more than about 50% of normal; and/or

(VI) (a) the use achieves circulatory factor IX protein or coagulation activity levels of less than about 300%, less than about 250%, less than about 200%, or less than about 150% of normal; and/or (b) the use increases circulatory factor IX protein or coagulation activity levels over the subject's baseline factor IX protein or coagulation activity levels by at least about 1%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%; and/or

(VII) the circulatory factor IX protein or coagulation activity levels are sustained for at least about 1 month, at least about 2 months, at least about 3 months, at least about 6 months, at least about 1 year, or at least about 2 years after the composition is to be administered,

optionally wherein the expression or activity of the factor IX protein is at least 50% or 60% of the expression or activity of the factor IX protein at a peak level of expression measured for the human subject at six months after the composition is to be administered,

optionally wherein the expression or activity of the factor IX protein is at least 50% or 60% of the expression or activity of the factor IX protein at a peak level of expression measured for the human subject at one year after the composition is to be administered, or

optionally wherein the expression or activity of the factor IX protein is at least 50% or 60% of the expression or activity of the factor IX protein at a peak level of expression measured for the human subject at two years after the composition is to be administered.

49. The composition for use according to any one of claims 43-48, wherein the use further comprises assessing preexisting AAV immunity in the subject prior to the composition is to be administered to the subject, optionally wherein the preexisting AAV immunity is preexisting AAV8 immunity, and optionally wherein assessing preexisting AAV immunity comprises assessing immunogenicity using a total antibody immune assay or a neutralizing antibody assay.

50. The composition for use according to any one of claims 40-49, wherein the nuclease agent comprises: (a) a Cas protein or a nucleic acid encoding the Cas protein; and (b) a guide RNA or one or more DNAs encoding the guide RNA, wherein the guide RNA comprises a DNA-targeting segment that targets a guide RNA target sequence, and wherein the guide RNA binds to the Cas protein and targets the Cas protein to the guide RNA target sequence, optionally wherein the guide RNA target sequence is in intron 1 of an *albumin* gene, wherein:

(I) the nucleic acid construct, the Cas protein or the nucleic acid encoding the Cas protein, and the guide RNA or the one or more DNAs encoding the guide RNAs are to be administered simultaneously; or

(II) the nucleic acid construct is not to be administered simultaneously with the Cas protein or the nucleic acid encoding the Cas protein and the guide RNA or the one or more DNAs encoding the guide RNAs.