We claim:

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

wherein the first factor IX protein coding sequence encodes a factor IX protein comprising the sequence set forth in SEQ ID NO: 195, and the second factor IX protein coding sequence encodes a factor IX protein comprising the sequence set forth in SEQ ID NO: 195, and wherein:

(I) the first factor IX protein coding sequence is at least 99% identical to the sequence set forth in SEQ ID NO: 159 and the second factor IX protein coding sequence is at least 99% identical to the sequence set forth in SEQ ID NO: 166; or

(II) the first factor IX protein coding sequence is at least 99% identical to the sequence set forth in SEQ ID NO: 166 and the second factor IX protein coding sequence is at least 99% identical to the sequence set forth in SEQ ID NO: 159.

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

(I) is not a hyperactive factor IX variant; and/or

(II) has all but one CpG dinucleotides removed or is fully CpG depleted; and

wherein the second factor IX protein coding sequence:

(I) is not a hyperactive factor IX variant; and/or

(II) has all but one CpG dinucleotides removed or is fully CpG depleted.

3. The composition of claim 1 or 2, 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.

4. The composition of any preceding claim, wherein:

(I) the first factor IX protein coding sequence comprises the sequence set forth in SEQ ID NO: 159 and the second factor IX protein coding sequence comprises the sequence set forth in SEQ ID NO: 166; or

(II) the first factor IX protein coding sequence comprises the sequence set forth in SEQ ID NO: 166 and the second factor IX protein coding sequence comprises the sequence set forth in SEQ ID NO: 159.

5. The composition of any preceding claim, wherein:

(I) the first factor IX protein coding sequence consists of the sequence set forth in SEQ ID NO: 159 and the second factor IX protein coding sequence consists of the sequence set forth in SEQ ID NO: 166; or

(II) the first factor IX protein coding sequence consists of the sequence set forth in SEQ ID NO: 166 and the second factor IX protein coding sequence consists of the sequence set forth in SEQ ID NO: 159.

6. The composition of any preceding claim, wherein the bidirectional nucleic acid construct comprises a splice acceptor upstream of the first factor IX protein coding sequence, and the bidirectional 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 bidirectional nucleic acid construct does not comprise homology arms, and wherein the bidirectional nucleic acid construct does not comprise a promoter that drives expression of the factor IX protein.

8. The composition of any preceding claim, wherein the bidirectional 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 bidirectional 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.

9. The composition of any preceding claim, wherein the bidirectional 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 the sequence set forth in SEQ ID NO: 159 and the second factor IX protein coding sequence comprises the sequence set forth in SEQ ID NO: 166; or (ii) the first factor IX protein coding sequence comprises the sequence set forth in SEQ ID NO: 166 and the second factor IX protein coding sequence comprises the sequence set forth in SEQ ID NO: 159,

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

wherein the bidirectional nucleic acid construct does not comprise homology arms.

10. The composition of any preceding claim, wherein the bidirectional nucleic acid construct comprises the sequence set forth in SEQ ID NO: 210 or 180 or the reverse complement thereof.

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

(i) the bidirectional nucleic acid construct is flanked by inverted terminal repeats (ITRs) on each end, wherein the ITR on at least one end comprises the sequence set forth in SEQ ID NO: 198, wherein the ITR on each end comprises the sequence set forth in SEQ ID NO: 198, wherein the ITR on at least one end comprises the sequence set forth in SEQ ID NO: 196, or wherein the ITR on each end comprises the sequence set forth in SEQ ID NO: 196; and/or

(ii) the AAV vector is a single-stranded AAV (ssAAV) vector; and/or

(iii) the AAV vector is a recombinant AAV8 (rAAV8) vector; and/or

(iv) the AAV vector is a single-stranded rAAV8 vector.

12. The composition of any preceding claim, wherein the bidirectional 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 the sequence set forth in SEQ ID NO: 159 and the second factor IX protein coding sequence comprises the sequence set forth in SEQ ID NO: 166; or (ii) the first factor IX protein coding sequence comprises the sequence set forth in SEQ ID NO: 166 and the second factor IX protein coding sequence comprises the sequence set forth in SEQ ID NO: 159,

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

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

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

optionally wherein the bidirectional nucleic acid construct is flanked by inverted terminal repeats (ITRs) on each end, wherein the ITR on at least one end comprises the sequence set forth in SEQ ID NO: 198, wherein the ITR on each end comprises the sequence set forth in SEQ ID NO: 198, wherein the ITR on at least one end comprises the sequence set forth in SEQ

ID NO: 196, or wherein the ITR on each end comprises the sequence set forth in SEQ ID NO: 196.

13. A cell comprising the composition of any one of claims 1-12.

14. The cell of claim 13, wherein the bidirectional 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 bidirectional 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.

15. The cell of claim 13 or 14, 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*.

16. Use of the composition of any one of claims 1-12 in the manufacture of a medicament for introducing a factor IX nucleic acid into a cell.

17. Use of the composition of any one of claims 1-12 in the manufacture of a medicament for integrating a factor IX bidirectional nucleic acid construct into a target gene in a cell.

18. Use of the composition of any one of claims 1-12 in the manufacture of a medicament for expressing factor IX in a cell.

19. The use of any one of claims 16-18, 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*.

20. Use of the composition of any one of claims 1-12 in the manufacture of a medicament for treating a factor IX deficiency in a subject.

21. Use of the composition of any one of claims 1-12 in the manufacture of a medicament for treating hemophilia B in a subject.

22. Use of the composition of any one of claims 1-12 in the manufacture of a medicament for preventing or inhibiting spontaneous bleeding in a subject having hemophilia B.

23. The use of any one of claims 20-22, 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.

24. The use of any one of claims 20-23, 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 1 μg/mL, at least 2 μg/mL, at least 3 μg/mL, at least 4 μg/mL, or at least 5 μg/mL, or wherein the use results in serum levels of the factor IX protein in the subject of between 1 μg/mL and 10 μg/mL; and/or

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

(b) the subject has moderate hemophilia, and the use achieves circulatory factor IX protein or coagulation activity levels of at least 5% or more than 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 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, more than 10%, more than 15%, more than 20%, more than 25%, more than 30%, more than 35%, more than 40%, more than 45%, or more than 50% of normal; and/or

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

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

25. The use of any one of claims 20-24, 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 assessing preexisting AAV immunity comprises assessing immunogenicity using a total antibody immune assay or a neutralizing antibody assay.

26. The use of claim 25, wherein the preexisting AAV immunity is preexisting AAV8 immunity.

27. The use of any one of claims 16-26, wherein the medicament further comprises a nuclease agent that targets a nuclease target site in a target gene, wherein the target gene is an *albumin* gene, optionally wherein the *albumin* gene is a human *albumin* gene.

28. The use of claim 27, 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.

29. The use of claim 27, 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.

30. The use of claim 29, wherein the DNA-targeting segment comprises any one of the sequences set forth in SEQ ID NOS: 30-61, or

wherein the guide RNA comprises any one of the sequences set forth in SEQ ID NOS: 62-125.

31. The use of claim 29 or 30, wherein the DNA-targeting segment comprises any one of the sequences set forth in SEQ ID NOS: 36, 30, 33, and 41, or

wherein the guide RNA comprises any one of the sequences set forth in SEQ ID NOS: 68, 100, 62, 94, 65, 97, 73, and 105.

32. The use of any one of claims 29-31, wherein the DNA-targeting segment comprises the sequence set forth in SEQ ID NO: 36, or wherein the DNA-targeting segment consists of the sequence set forth in SEQ ID NO: 36.

33. The use of any one of claims 29-32, wherein the guide RNA comprises the sequence set forth in SEQ ID NO: 68 or 100, wherein the composition comprises the guide RNA in the form of RNA.

34. The use of any one of claims 29-33, wherein the composition comprises the guide RNA in the form of RNA, the guide RNA comprises the sequence set forth in 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.

35. The use of any one of claims 29-34, wherein the Cas protein is a Cas9 protein, optionally wherein the Cas protein is derived from a *Streptococcus pyogenes* Cas9 protein.

36. The use of any one of claims 29-35, wherein the Cas protein comprises the sequence set forth in SEQ ID NO: 11.

37. The use of any one of claims 29-36, 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.

38. The use of claim 37, wherein the mRNA encoding the Cas protein comprises the sequence set forth in SEQ ID NO: 226 or 225.

39. The use of any one of claims 29-38, 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.

40. The use of any one of claims 29-38, wherein the composition comprises the guide RNA in the form of RNA, and the guide RNA comprises the sequence set forth in 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.

41. The use of any one of claims 29-38, wherein the composition comprises the guide RNA in the form of RNA, the guide RNA comprises the sequence set forth in 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.

42. The use of claim 40 or 41, wherein the bidirectional 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 the sequence set forth in SEQ ID NO: 159 and the second factor IX protein coding sequence comprises the sequence set forth in SEQ ID NO: 166; or (ii) the first factor IX protein coding sequence comprises the sequence set forth in SEQ ID NO: 166 and the second factor IX protein coding sequence comprises the sequence set forth in SEQ ID NO: 159,

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

wherein the bidirectional nucleic acid construct does not comprise homology arms.

43. The use of any one of claims 29-42, 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,

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

44. The use of claim 43, wherein the cationic lipid is Lipid A, the neutral lipid is DSPC, the helper lipid is cholesterol, and the stealth lipid is 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.

45. The use of any one of claims 29-44, 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 the sequence set forth in 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.

46. The use of any one of claims 29-44, 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 the sequence set forth in 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.

47. The use of claim 45 or 46, wherein the bidirectional 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 the bidirectional nucleic acid construct does not comprise a promoter that drives expression of the factor IX protein,

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

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

optionally wherein the bidirectional nucleic acid construct is flanked by inverted terminal repeats (ITRs) on each end, wherein the ITR on at least one end comprises the sequence set forth in SEQ ID NO: 198, wherein the ITR on each end comprises the sequence set forth in SEQ ID NO: 198, wherein the ITR on at least one end comprises the sequence set forth in SEQ ID NO: 196, or wherein the ITR on each end comprises the sequence set forth in SEQ ID NO: 196.

48. The use of any one of claims 29-47, wherein:

(I) the bidirectional 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 RNA are to be administered simultaneously; or

(II) the bidirectional 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 RNA.