where the gene cassette remains almost exclusively episomal and it is the episomal copies of the therapeutic gene that give rise to the therapeutic protein. AAV does not have a mechanism to integrate its encapsulated DNA into the genome of the host cells but instead is maintained as an episome that is therefore not replicated when the host cell divides. Episomal DNA can also be subject to degradation over time. It has been demonstrated that when liver cells containing AAV episomes are induced to divide, the AAV genome is not replicated but is instead diluted. As a result, AAV based gene therapy is not expected to be effective when given to children whose livers have not yet achieved adult size. In addition, it is currently unknown how long a AAV based gene therapy will persist when given to adult humans, although animal data have demonstrated only small losses in therapeutic effect over periods as long as 10 years. Therefore, there is a critical need for developing new effective and permeant treatments for HemA.

SUMMARY

[0005a] In one aspect, the present disclosure refers to a system comprising: a deoxyribonucleic acid (DNA) endonuclease or nucleic acid encoding said DNA endonuclease; a guide RNA (gRNA) comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, 30, 18-20, 23-27, 29, 31-44, and 104; and a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof.

[0005b] In another aspect, the present disclosure refers to an *ex vivo* method of editing a genome in a cell, the method comprising: providing the following to the cell: (a) a gRNA comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, 30, 18-20, 23-27, 29, 31-44, and 104; (b) a DNA endonuclease or nucleic acid encoding said DNA endonuclease; and (c) a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative.

[0005c] In another aspect, the present disclosure refers to the use of the following: (a) a gRNA comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, 30, 18-20, 23-27, 29, 31-44, and 104; (b) a DNA endonuclease or nucleic acid encoding said DNA endonuclease; and (c) a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative in the manufacture of a medicament for combination therapy to treat Hemophilia A in a subject.

[0005d] In another aspect, the present disclosure refers to the use of the genetically modified cell as described herein in the manufacture of a medicament for treating Hemophilia A, wherein said medicament is to be administered to the subject.

[0006] In one example, provided herein is a guide RNA (gRNA) sequence having a sequence that is complementary to a genomic sequence within or near an endogenous albumin locus.

[0007] In some examples, the gRNA comprises a spacer sequence selected from those listed in Table 3 and variants thereof having at least 85% homology to any of those listed in Table 3.

[0008] In another example, provided herein is a composition having any of the above-mentioned gRNAs.

[0009] In some examples, the gRNA of the composition comprises a spacer sequence selected from those listed in Table 3 and variants thereof having at least 85% homology to any of those listed in Table 3.

[0010] In some examples, the composition further comprises one or more of the following: a deoxyribonucleic acid (DNA) endonuclease or a nucleic acid encoding the DNA endonuclease; and a donor template having a nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof.

[0011] In some examples, the DNA endonuclease is selected from the group consisting of a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease, or a functional derivative thereof.

[0012] In some examples, the DNA endonuclease is Cas9. In some examples, the Cas9 is from *Streptococcus pyogenes* (spCas9). In some examples, the Cas9 is from *Staphylococcus lugdunensis* (SluCas9).

[0013] In some examples, the nucleic acid encoding the DNA endonuclease is codon optimized.

[0014] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof is codon optimized.

[0015] In some examples, the nucleic acid encoding the DNA endonuclease is a deoxyribonucleic acid (DNA).

[0016] In some examples, the nucleic acid encoding the DNA endonuclease is a ribonucleic acid (RNA).

[0017] In some examples, the RNA encoding the DNA endonuclease is linked to the gRNA via a covalent bond.

[0018] In some examples, the composition further comprises a liposome or lipid nanoparticle.

[0019] In some examples, the donor template is encoded in an Adeno Associated Virus (AAV) vector.

[0020] In some examples, the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

[0021] In some examples, the liposome or lipid nanoparticle also comprises the gRNA.

[0022] In some examples, the DNA endonuclease is precomplexed with the gRNA, forming a Ribonucleoprotein (RNP) complex.

[0023] In another example, provided herein is a kit having any of the compositions described above and further having instructions for use.

[0024] In another example, provided herein is a system comprising a deoxyribonucleic acid (DNA) endonuclease or nucleic acid encoding said DNA endonuclease; a guide RNA (gRNA) comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, 30, 18-20, 23-27, 29, 31-44, and 104; and a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof.

[0025] In some examples, the gRNA comprises a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, and 30. In some examples, the gRNA comprises a spacer sequence from SEQ ID NO: 22. In some examples, the gRNA comprises a spacer sequence from SEQ ID NO: 21. In some examples, the gRNA comprises a spacer sequence from SEQ ID NO: 28. In some examples, the gRNA comprises a spacer sequence from SEQ ID NO: 30.

[0026] In some examples, the DNA endonuclease is selected from the group consisting of a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease, or a functional derivative thereof. In some examples, the DNA endonuclease is Cas9. In some examples, the Cas9 is from *Streptococcus pyogenes* (spCas9). In some examples, the Cas9 is from *Staphylococcus lugdunensis* (SluCas9).

[0027] In some examples, the nucleic acid encoding said DNA endonuclease is codon optimized for expression is a host cell. In some examples, the host cell is a human cell.

[0028] In some examples, the nucleic acid encoding a Factor VIII (FVIII) protein or functional derivative thereof is codon optimized for expression in a host cell. In some examples, the host cell is a human cell.

[0029] In some examples, the nucleic acid encoding said DNA endonuclease is a deoxyribonucleic acid (DNA).

[0030] In some examples, the nucleic acid encoding said DNA endonuclease is a ribonucleic acid (RNA). In some examples, the RNA encoding said DNA endonuclease is an mRNA.

[0031] In some examples, the donor template is encoded in an Adeno Associated Virus (AAV) vector.

[0032] In some examples, the donor template comprises a donor cassette comprising the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative, and the donor cassette is flanked on one or both sides by a gRNA target site. In some examples, the donor cassette is flanked on both sides by a gRNA target site. In some examples, the gRNA target site is a target site for a gRNA in the system. In some examples, the gRNA target site of the donor template is the reverse complement of a genomic gRNA target site for a gRNA in the system.

[0033] In some examples, the DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle. In some examples, the liposome or lipid nanoparticle also comprises the gRNA.

[0034] In someexample, the system comprises the DNA endonuclease precomplexed with the gRNA, forming a Ribonucleoprotein (RNP) complex.

[0035] In another example, provided herein is a method of editing a genome in a cell, the method comprising providing the following to the cell: (a) any of the gRNAs described above; (b) a deoxyribonucleic acid (DNA) endonuclease or a nucleic acid encoding the DNA endonuclease; and (c) a donor template having a nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative.

[0036] In some examples, the gRNA comprises a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, 30, 18-20, 23-27, 29, 31-44, and 104. In some example, the gRNA comprises a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, and 30. In some examples, the gRNA comprises a spacer sequence from SEQ ID NO: 22. In some examples, the gRNA comprises a spacer sequence from SEQ ID NO: 21. In some examples, the gRNA comprises a spacer sequence from SEQ ID NO: 28. In some examples, the gRNA comprises a spacer sequence from SEQ ID NO: 30.

[0037] In some examples, the gRNA has a spacer sequence selected from those listed in Table 3 and variants thereof having at least 85% homology to any of those listed in Table 3.

[0038] In some examples, the DNA endonuclease is selected from the group consisting of a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease; or a functional derivative thereof.

[0039] In some examples, the DNA endonuclease is Cas9. In some examples, the Cas9 is from *Streptococcus pyogenes* (spCas9). In some examples, the Cas9 is from *Staphylococcus lugdunensis* (SluCas9).

[0040] In some examples, the nucleic acid encoding the DNA endonuclease is codon optimized for expression in the cell.

[0041] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof is codon optimized for expression in the cell.

[0042] In some examples, the nucleic acid encoding the DNA endonuclease is a deoxyribonucleic acid (DNA).

[0043] In some examples, the nucleic acid encoding the DNA endonuclease is a ribonucleic acid (RNA).

[0044] In some examples, the RNA encoding said DNA endonuclease is an mRNA.

[0045] In some examples, the RNA encoding the DNA endonuclease is linked to the gRNA via a covalent bond.

[0046] In some examples, the donor template is encoded in an Adeno Associated Virus (AAV) vector.

[0047] In some examples, the donor template comprises a donor cassette comprising the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative, and the donor cassette is flanked on one or both sides by a gRNA target site. In some examples, the donor cassette is flanked on both sides by a gRNA target site. In some examples, the gRNA target site is a target site for the gRNA of (a). In some examples, the gRNA target site of the donor template is the reverse complement of a gRNA target site in the cell genome for the gRNA of (a). In some example,

[0048] In some examples, one or more of (a), (b) and (c) are formulated in a liposome or lipid nanoparticle.

[0049] In some examples, the DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

[0050] In some examples, the liposome or lipid nanoparticle also comprises the gRNA.

[0051] In some examples, the DNA endonuclease is precomplexed with the gRNA, forming a Ribonucleoprotein (RNP) complex, prior to the provision to the cell.

[0052] In some examples, (a) and (b) are provided to the cell after (c) is provided to the cell.

[0053] In some examples, (a) and (b) are provided to the cell about 1 to 14 days after (c) is provided to the cell.

[0054] In some examples, the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell more than 4 days after the donor template of (c) is provided to the cell.

[0055] In some examples, the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell at least 14 days after (c) is provided to the cell.

[0056] In some examples, one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b).

[0057] In some examples, one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) until a target level of targeted integration of the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative and/or a target level of expression of the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is achieved.

[0058] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is inserted into a genomic sequence of the cell.

[0059] In some examples, the insertion is at, within, or near the albumin gene or albumin gene regulatory elements in the genome of the cell.

[0060] In some examples, the insertion is in the first intron of the albumin gene.

[0061] In some examples, the insertion is at least 37 bp downstream of the end of the first exon of the human albumin gene in the genome and at least 330 bp upstream of the start of the second exon of the human albumin gene in the genome.

[0062] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed under the control of the endogenous albumin promoter.

[0063] In some examples, the cell is a hepatocyte.

[0064] In another aspect, provided herein is a genetically modified cell in which the genome of the cell is edited by any of the method described above.

[0065] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is inserted into a genomic sequence of the cell.

[0066] In some examples, the insertion is at, within, or near the albumin gene or albumin gene regulatory elements in the genome of the cell.

[0067] In some examples, the insertion is in the first intron of the albumin gene.

[0068] In some examples, the insertion is at least 37 bp downstream of the end of the first exon of the human albumin gene in the genome and at least 330 bp upstream of the start of the second exon of the human albumin gene in the genome.

[0069] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed under the control of the endogenous albumin promoter.

[0070] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof is codon optimized.

[0071] In some examples, the cell is a hepatocyte.

[0072] In another example, provided herein is a method of treating Hemophilia A in a subject, the method comprising providing the following to a cell in the subject: (a) a gRNA comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, 30, 18-20, 23-27, 29, 31-44, and 104; (b) a DNA endonuclease or nucleic acid encoding said DNA endonuclease; and (c) a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative.

[0073] In some examples, the gRNA comprises a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, and 30. In some examples, the gRNA comprises a spacer sequence from SEQ ID NO: 22. In some examples, the gRNA comprises a spacer sequence from SEQ ID NO: 21. In some examples, the gRNA comprises a spacer sequence from SEQ ID NO: 28. In some examples, the gRNA comprises a spacer sequence from SEQ ID NO: 30.

[0074] In some examples, the subject is a patient having or is suspected of having Hemophilia A.

[0075] In some examples, the subject is diagnosed with a risk of Hemophilia A.

[0076] In some examples, the DNA endonuclease is selected from the group consisting of a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease; or a functional derivative thereof.

[0077] In some examples, the DNA endonuclease is Cas9. In some examples, the Cas9 is from *Streptococcus pyogenes* (spCas9). In some examples, the Cas9 is from *Staphylococcus lugdunensis* (SluCas9).

[0078] In some examples, the nucleic acid encoding said DNA endonuclease is codon optimized for expression in the cell.

[0079] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof is codon optimized for expression in the cell.

[0080] In some examples, the nucleic acid encoding said DNA endonuclease is a deoxyribonucleic acid (DNA).

[0081] In some examples, the nucleic acid encoding said DNA endonuclease is a ribonucleic acid (RNA). In some examples, the RNA encoding said DNA endonuclease is an mRNA.

[0082] In some examples, one or more of the gRNA of (a), the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b), and the donor template of (c) are formulated in a liposome or lipid nanoparticle.

[0083] In some examples, the donor template is encoded in an Adeno Associated Virus (AAV) vector.

[0084] In some examples, the donor template comprises a donor cassette comprising the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative, and wherein the donor cassette is flanked on one or both sides by a gRNA target site. In some examples, the donor cassette is flanked on both sides by a gRNA target site. In some examples, the gRNA target site is a target site for the gRNA of (a). In some examples, the gRNA target site of the donor template is the reverse complement of the gRNA target site in the cell genome for the gRNA of (a).

[0085] In some examples, providing the donor template to the cell comprises administering the donor template to the subject. In some examples, the administration is via intravenous route.

[0086] In some examples, DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle. In some examples, the liposome or lipid nanoparticle also comprises the gRNA.

[0087] In some examples, providing the gRNA and the DNA endonuclease or nucleic acid encoding the DNA endonuclease to the cell comprises administering the liposome or lipid nanoparticle to the subject. In some examples, the administration is via intravenous route.

[0088] In some examples, the method comprises providing to the cell the DNA endonuclease pre-complexed with the gRNA, forming a Ribonucleoprotein (RNP) complex.

[0089] In some examples, the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell more than 4 days after the donor template of (c) is provided to the cell. In some examples, the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell at least 14 days after the donor template of (c) is provided to the cell.

[0090] In some examples, one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b). In some examples, one or more additional doses of the gRNA of

(a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) until a target level of targeted integration of the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative and/or a target level of expression of the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is achieved.

[0091] In some examples, providing the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) to the cell comprises administering to the subject a lipid nanoparticle comprising nucleic acid encoding the DNA endonuclease and the gRNA.

[0092] In some examples, providing the donor template of (c) to the cell comprises administering to the subject the donor template encoded in an AAV vector.

[0093] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed under the control of the endogenous albumin promoter.

[0094] In some examples, the cell is a hepatocyte.

[0095] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed in the liver of the subject.

[0096] In another example, provided herein is a method of treating Hemophilia A in a subject. The method comprises administering any of the above-mentioned genetically modified cells to the subject.

[0097] In some examples, the subject is a patient having or is suspected of having Hemophilia A.

[0098] In some examples, the subject is diagnosed with a risk of Hemophilia A.

[0099] In some examples, the genetically modified cell is autologous.

[0100] In some examples, the cell is a hepatocyte.

[0101] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is inserted into a genomic sequence of the cell.

[0102] In some examples, the insertion is at, within, or near the albumin gene or albumin gene regulatory elements in the genome of the cell.

[0103] In some examples, the insertion is in the first intron of the albumin gene.

[0104] In some examples, the insertion is at least 37 bp downstream of the end of the first exon of the human albumin gene in the genome and at least 330 bp upstream of the start of the second exon of the human albumin gene in the genome.

[0105] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed under the control of the endogenous albumin promoter.

[0106] In some examples, the method further comprises obtaining a biological sample from the subject wherein the biological sample comprises a hepatocyte cell and editing the genome of the hepatocyte cell by inserting a nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof into a genomic sequence of the cell, thereby producing the genetically modified cell.

[0107] In another example, provided herein is a method of treating Hemophilia A in a subject. The method comprises obtaining a biological sample from the subject wherein the biological sample comprises a hepatocyte cell, providing the following to the hepatocyte cell: (a) any of the gRNA described above; (b) a deoxyribonucleic acid (DNA) endonuclease or a nucleic acid encoding the DNA endonuclease; and (c) a donor template having a nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative, thereby producing a genetically modified cell, and administering the genetically modified cell to the subject.

[0108] In some examples, the subject is a patient having or is suspected of having Hemophilia A.

[0109] In some examples, the subject is diagnosed with a risk of Hemophilia A.

[0110] In some examples, the genetically modified cell is autologous.

[0111] In some examples, the gRNA comprises a sequence selected from those listed in Table 3 and variants thereof having at least 85% homology to any of those listed in Table 3.

[0112] In some examples, the DNA endonuclease is selected from the group consisting of a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease, or a functional derivative thereof.

[0113] In some examples, the DNA endonuclease is Cas9. In some examples, the Cas9 is from *Streptococcus pyogenes* (spCas9). In some examples, the Cas9 is from *Staphylococcus lugdunensis* (SluCas9).

[0114] In some examples, the nucleic acid encoding the DNA endonuclease is codon optimized.

[0115] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof is codon optimized.

[0116] In some examples, the nucleic acid encoding the DNA endonuclease is a deoxyribonucleic acid (DNA) sequence.

[0117] In some examples, the nucleic acid encoding the DNA endonuclease is a ribonucleic acid (RNA) sequence.

[0118] In some examples, the RNA sequence encoding the DNA endonuclease is linked to the gRNA via a covalent bond.

[0119] In some examples, one or more of (a), (b) and (c) are formulated in a liposome or lipid nanoparticle.

[0120] In some examples, the donor template is encoded in an Adeno Associated Virus (AAV) vector.

[0121] In some examples, the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

[0122] In some examples, the liposome or lipid nanoparticle also comprises the gRNA.

[0123] In some examples, the DNA endonuclease is precomplexed with the gRNA, forming a Ribonucleoprotein (RNP) complex, prior to the provision to the cell.

[0124] In some examples, (a) and (b) are provided to the cell after (c) is provided to the cell.

[0125] In some examples, (a) and (b) are provided to the cell about 1 to 14 days after (c) is provided to the cell.

[0126] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is inserted into a genomic sequence of the cell.

[0127] In some examples, the insertion is at, within, or near the albumin gene or albumin gene regulatory elements in the genome of the cell.

[0128] In some examples, the insertion is in the first intron of the albumin gene.

[0129] In some examples, the insertion is at least 37 bp downstream of the end of the first exon of the human albumin gene in the genome and at least 330 bp upstream of the start of the second exon of the human albumin gene in the genome.

[0130] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed under the control of the endogenous albumin promoter.

[0131] In some examples, the cell is a hepatocyte.

[0132] In another example, provided herein is a method of treating Hemophilia A in a subject. The method comprises providing the following to a cell in the subject: (a) any of the gRNA described above; (b) a deoxyribonucleic acid (DNA) endonuclease or a nucleic acid encoding the DNA endonuclease; and (c) a donor template having a nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative.

[0133] In some examples, the subject is a patient having or is suspected of having Hemophilia A.

[0134] In some examples, the subject is diagnosed with a risk of Hemophilia A.

[0135] In some examples, the gRNA comprises a sequence selected from those listed in Table 3 and variants thereof having at least 85% homology to any of those listed in Table 3.

[0136] In some examples, the DNA endonuclease is selected from the group consisting of a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease; or a functional derivative thereof.

[0137] In some examples, the DNA endonuclease is Cas9. In some examples, the Cas9 is from *Streptococcus pyogenes* (spCas9). In some examples, the Cas9 is from *Staphylococcus lugdunensis* (SluCas9).

[0138] In some examples, the nucleic acid encoding the DNA endonuclease is codon optimized.

[0139] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof is codon optimized.

[0140] In some examples, the nucleic acid encoding the DNA endonuclease is a deoxyribonucleic acid (DNA) sequence.

[0141] In some examples, the nucleic acid encoding the DNA endonuclease is a ribonucleic acid (RNA) sequence.

[0142] In some examples, the RNA sequence encoding the DNA endonuclease is linked to the gRNA via a covalent bond.

[0143] In some examples, one or more of (a), (b) and (c) are formulated in a liposome or lipid nanoparticle.

[0144] In some examples, the donor template is encoded in an Adeno Associated Virus (AAV) vector.

[0145] In some examples, the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

[0146] In some examples, the liposome or lipid nanoparticle also comprises the gRNA.

[0147] In some examples, the DNA endonuclease is precomplexed with the gRNA, forming a Ribonucleoprotein (RNP) complex, prior to the provision to the cell.

[0148] In some examples, (a) and (b) are provided to the cell after (c) is provided to the cell.

[0149] In some examples, (a) and (b) are provided to the cell about 1 to 14 days after (c) is provided to the cell.

[0150] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is inserted into a genomic sequence of the cell.

[0151] In some examples, the insertion is at, within, or near the albumin gene or albumin gene regulatory elements in the genome of the cell.

[0152] In some examples, the insertion is in the first intron of the albumin gene in the genome of the cell.

[0153] In some examples, the insertion is at least 37 bp downstream of the end of the first exon of the human albumin gene in the genome and at least 330 bp upstream of the start of the second exon of the human albumin gene in the genome.

[0154] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed under the control of the endogenous albumin promoter.

[0155] In some examples, the cell is a hepatocyte.

[0156] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed in the liver of the subject.

[0157] In another aspect, provided herein is a kit comprising one or more elements of a system described above, and further comprising instructions for use.

WHAT IS CLAIMED IS:

1. A system comprising:

a deoxyribonucleic acid (DNA) endonuclease or nucleic acid encoding said DNA endonuclease;

a guide RNA (gRNA) comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, 30, 18-20, 23-27, 29, 31-44, and 104; and

a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof.

2. The system of claim 1, wherein the gRNA comprises a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, and 30.

3. The system of claim 2, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 22.

4. The system of claim 2, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 21.

5. The system of claim 2, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 28.

6. The system of claim 2, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 30.

7. The system of any one of claims 1-6, wherein said DNA endonuclease is selected from the group consisting of a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease, or a functional derivative thereof.

8. The system of any one of claims 1-7, wherein said DNA endonuclease is Cas9.

9. The system of any one of claims 1-8, wherein the nucleic acid encoding said DNA endonuclease is codon optimized for expression in a host cell.

10. The system of any one of claims 1-9, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof is codon optimized for expression in a host cell.

11. The system of any one of claims 1-10, wherein the nucleic acid encoding said DNA endonuclease is a deoxyribonucleic acid (DNA).

12. The system of any one of claims 1-10, wherein the nucleic acid encoding said DNA endonuclease is a ribonucleic acid (RNA).

13. The system of claim 12, wherein the RNA encoding said DNA endonuclease is an mRNA.

14. The system of any one of claims 1-13, wherein the donor template is encoded in an Adeno Associated Virus (AAV) vector.

15. The system of claim 14, wherein the donor template comprises a donor cassette comprising the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative, and wherein the donor cassette is flanked on one or both sides by a gRNA target site.

16. The system of claim 15, wherein the donor cassette is flanked on both sides by a gRNA target site.

17. The system of claim 15 or 16, wherein the gRNA target site is a target site for a gRNA in the system.

18. The system of claim 17, wherein the gRNA target site of the donor template is the reverse complement of a genomic gRNA target site for a gRNA in the system.

19. The system of any one of claims 1-18, wherein said DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

20. The system of claim 19, wherein said liposome or lipid nanoparticle also comprises the gRNA.

21. The system of any one of claims 1-20, comprising the DNA endonuclease precomplexed with the gRNA, forming a Ribonucleoprotein (RNP) complex.

22. An *ex vivo* method of editing a genome in a cell, the method comprising: providing the following to the cell:

(a) a gRNA comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, 30, 18-20, 23-27, 29, 31-44, and 104;

(b) a DNA endonuclease or nucleic acid encoding said DNA endonuclease; and

(c) a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative.

23. The method of claim 22, wherein the gRNA comprises a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, and 30.

24. The method of claim 23, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 21.

25. The method of claim 23, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 22.

26. The method of claim 23, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 28.

27. The method of claim 23, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 30.

28. The method of any one of claims 22-27, wherein said DNA endonuclease is selected from the group consisting of a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1,

Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease; or a functional derivative thereof.

29. The method of any one of claims 22-28, wherein said DNA endonuclease is Cas9.

30. The method of any one of claims 22-29, wherein the nucleic acid encoding said DNA endonuclease is codon optimized for expression in the cell.

31. The method of any one of claims 22-30, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof is codon optimized for expression in the cell.

32. The method of any one of claims 22-31, wherein the nucleic acid encoding said DNA endonuclease is a deoxyribonucleic acid (DNA).

33. The method of any one of claims 22-31, wherein the nucleic acid encoding said DNA endonuclease is a ribonucleic acid (RNA).

34. The method of claim 33, wherein the RNA encoding said DNA endonuclease is an mRNA.

35. The method of any one of claims 22-34, wherein the donor template is encoded in an Adeno Associated Virus (AAV) vector.

36. The method of any one of claims 22-35, wherein the donor template comprises a donor cassette comprising the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative, and wherein the donor cassette is flanked on one or both sides by a gRNA target site.

37. The method of claim 36, wherein the donor cassette is flanked on both sides by a gRNA target site.

38. The method of claim 36 or 37, wherein the gRNA target site is a target site for the gRNA of (a).

39. The method of claim 38, wherein the gRNA target site of the donor template is the reverse complement of a gRNA target site in the cell genome for the gRNA of (a).

40. The method of any one of claims 22-39, wherein said DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

41. The method of claim 40, wherein said liposome or lipid nanoparticle also comprises the gRNA.

42. The method of any one of claims 22-41, comprising providing to the cell the DNA endonuclease precomplexed with the gRNA, forming a Ribonucleoprotein (RNP) complex.

43. The method of any one of claims 22-42, wherein the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell more than 4 days after the donor template of (c) is provided to the cell.

44. The method of any one of claims 22-43, wherein the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell at least 14 days after (c) is provided to the cell.

45. The method of claim 43 or 44, wherein one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b).

46. The method of claim 45, wherein one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) until a target level of targeted integration of the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative and/or a target level of expression of the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is achieved.

47. The method of any one of claims 22-46, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed under the control of the endogenous albumin promoter.

48. The method of any one of claims 22-47, wherein said cell is a hepatocyte.

49. A genetically modified cell in which the genome of the cell is edited by the method of any one of claims 22-48.

50. The genetically modified cell of claim 49, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed under the control of the endogenous albumin promoter.

51. The genetically modified cell of claim 49 or 50, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof is codon optimized for expression in the cell.

52. The genetically modified cell of any one of claims 49-51, wherein said cell is a hepatocyte.

53. Use of the following:

(a) a gRNA comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, 30, 18-20, 23-27, 29, 31-44, and 104;

(b) a DNA endonuclease or nucleic acid encoding said DNA endonuclease; and

(c) a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative

in the manufacture of a medicament for combination therapy to treat Hemophilia A in a subject.

54. The use of claim 53, wherein the gRNA comprises a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, and 30.

55. The use of claim 54, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 22.

56. The use of claim 54, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 21.

57. The use of claim 54, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 28.

58. The useof claim 54, wherein the gRNA comprises a spacer sequence from SEQ ID NO: 30.

59. The use of any one of claims 53-58, wherein said subject is a patient having or is suspected of having Hemophilia A.

60. The use of any one of claims 53-58, wherein said subject is diagnosed with a risk of Hemophilia A.

61. The use of any one of claims 53-60, wherein said DNA endonuclease is selected from the group consisting of a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease; or a functional derivative thereof.

62. The use of any one of claims 53-61, wherein said DNA endonuclease is Cas9.

63. The use of any one of claims 53-62, wherein the nucleic acid encoding said DNA endonuclease is codon optimized for expression in the cell.

64. The use of any one of claims 53-63, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative thereof is codon optimized for expression in the cell.

65. The use of any one of claims 53-64, wherein the nucleic acid encoding said DNA endonuclease is a deoxyribonucleic acid (DNA).

66. The use of any one of claims 53-64, wherein the nucleic acid encoding said DNA endonuclease is a ribonucleic acid (RNA).

67. The use of claim 66, wherein the RNA encoding said DNA endonuclease is an mRNA.

68. The use of any one of claims 53-67, wherein one or more of the gRNA of (a), the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b), and the donor template of (c) are formulated in a liposome or lipid nanoparticle.

69. The use of any one of claims 53-68, wherein the donor template is encoded in an Adeno Associated Virus (AAV) vector.

70. The use of any one of claims 53-69, wherein the donor template comprises a donor cassette comprising the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative, and wherein the donor cassette is flanked on one or both sides by a gRNA target site.

71. The use of claim 70, wherein the donor cassette is flanked on both sides by a gRNA target site.

72. The use of claim 70 or 71, wherein the gRNA target site is a target site for the gRNA of (a).

73. The use of claim 72, wherein the gRNA target site of the donor template is the reverse complement of the gRNA target site in the cell genome for the gRNA of (a).

74. The use of any one of claims 53-73, wherein providing the donor template to the cell comprises administration of the donor template to the subject.

75. The use of claim 74, wherein the administration is via intravenous route.

76. The use of any one of claims 53-75, wherein said DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

77. The use of claim 76, wherein said liposome or lipid nanoparticle also comprises the gRNA.

78. The use of claim 77, wherein providing the gRNA and the DNA endonuclease or nucleic acid encoding the DNA endonuclease to the cell comprises administration of the liposome or lipid nanoparticle to the subject.

79. The use of claim 78, wherein the administration is via intravenous route.

80. The use of any one of claims 53-79, comprising providing to the cell the DNA endonuclease pre-complexed with the gRNA, forming a Ribonucleoprotein (RNP) complex.

81. The use of any one of claims 53-80, wherein the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell more than 4 days after the donor template of (c) is provided to the cell.

82. The use of any one of claims 53-81, wherein the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell at least 14 days after the donor template of (c) is provided to the cell.

83. The use of claim 81 or 82, wherein one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b).

84. The use of claim 83, wherein one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) until a target level of targeted integration of the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative and/or a target level of expression of the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is achieved.

85. The use of any one of claims 81-84, wherein providing the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) to the cell

comprises administration to the subject a lipid nanoparticle comprising nucleic acid encoding the DNA endonuclease and the gRNA.

86. The use of any one of claims 81-85, wherein providing the donor template of (c) to the cell comprises administration to the subject the donor template encoded in an AAV vector.

87. The use of any one of claims 53-86, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed under the control of the endogenous albumin promoter.

88. The use of any one of claims 53-87, wherein said cell is a hepatocyte.

89. The use of any one of claims 53-88, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed in the liver of the subject.

90. Use of the genetically modified cell of any one of claims 49-52 in the manufacture of a medicament for treating Hemophilia A, wherein said medicament is to be administered to the subject.

91. The use of claim 90, wherein said genetically modified cell is autologous to the subject.

92. The use of claim 90 or 91 further comprising:

a biological sample obtained from the subject wherein the biological sample comprises a hepatocyte cell, wherein the genetically modified cell is prepared from the hepatocyte.

93. A kit comprising one or more elements of the system of any one of claims 1-21, and further comprising instructions for use.