multiple ways, such as disrupting gene function, repairing a mutant gene to normal, and inserting DNA material.

[0006] However, presently available genome editing techniques can suffer from a number of disadvantages, for example, in accuracy, unacceptable levels of off-target editing, etc. Accordingly, there remains a need for new genome gene editing platforms that are capable of manipulating genes at specific genomic loci in multiple ways, such as disrupting gene function, repairing a mutant gene to normal, and/or inserting heterologous DNA material at specific loci, such as a safe harbor locus within the genome of a target cell.

SUMMARY

[0007] Provided herein, *inter alia*, are compositions, methods, and systems for targeted delivery of nucleic acids, including DNA and RNA, to a target cell, e.g., human cell. Also provided are compositions, methods, and systems for expressing a transgene in a cell by integration of the transgene into the genome of the cell in a targeted manner by genomic editing. Certain examples and examples of the disclosure concern compositions, methods, and systems for knocking in a gene-of-interest (GOI) into a specific safe habor location in the genome, in particular to a genomic location within or near an endogenous albumin locus. Further provided are compositions, methods, and systems for treating a subject having or suspected of having a disorder or health condition employing *ex vivo* and/or *in vivo* genome editing.

[0007a] In one aspect, the present disclosure refers to a system comprising: a Cas9 endonuclease or nucleic acid encoding said Cas9 endonuclease; a guide RNA (gRNA) comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, and 30, or nucleic acid encoding the gRNA; and a donor template comprising a nucleic acid sequence encoding a protein selected from the group consisting of Factor IX (FIX) protein, alpha-1-antitrypsin, Factor XIII (FXIII) protein, Factor VII (FVII) protein, Factor X (FX) protein, Protein C, and serine protease inhibitor G1 (serpin G1).

[0008] 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.

[0009] In some examples, the gRNA has 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.

[0010] In another example, provided herein is a composition having any of the above-mentioned gRNA.

[0011] In one example, provided herein is a system including a guide RNA (gRNA) as disclosed herein or nucleic acid encoding the gRNA. In some examples, the gRNA of the system has 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.

[0012] In some examples, the system further has 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 gene-of-interest (GOI) or functional derivative thereof. 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

[0024] In some examples, the DNA endonuclease is precomplexed with the gRNA, forming a ribonucleoprotein (RNP) complex.

[0025] In another example, provided herein is a kit having any of the composition described above and further having instructions for use.

[0025a] In a further aspect, the present disclosure refers to a method of editing a genome in a cell, the method comprising: providing the following to the cell: a gRNA comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, and 30, or nucleic acid encoding the gRNA; a Cas9 endonuclease or nucleic acid encoding the Cas9 endonuclease; and a donor template comprising a nucleic acid sequence encoding a protein selected from the group consisting of Factor IX (FIX) protein, alpha-1-antitrypsin, Factor XIII (FXIII) protein, Factor VII (FVII) protein, Factor X (FX) protein, Protein C, and serine protease inhibitor G1 (serpin G1), wherein the method is not a method for treatment of the human or animal body by therapy. In yet another aspect, the present disclosure refers to a genetically modified cell in which the genome of the cell is edited by a method as disclosed herein.

[0026] In another example, provided herein is a method of editing a genome in a cell. The method includes providing the following to the cell: (a) any of the gRNA described herein or nucleic acid encoding the gRNA; (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 gene-of-interest (GOI) or functional derivative. 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: 21. 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: 28. In some examples, the gRNA comprises a spacer sequence from SEQ ID NO: 30.

[0027] In some examples, the gRNA has 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.

[0028] In some examples, the DNA endonuclease recognizes a protospacer adjacent motif (PAM) having the sequence NGG or NNGG, wherein N is any nucleotide; or a functional derivative thereof. In some examples, the DNA endonuclease is a type II Cas endonuclease or a functional derivative thereof.

[0029] 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).

[0030] In some examples, the nucleic acid encoding the DNA endonuclease is codon optimized.

[0031] In some examples, the nucleic acid sequence encoding the gene-of-interest (GOI) is codon optimized. In some examples, the GOI encodes a polypeptide selected from the group consisting of a therapeutic polypeptide and a prophylactic polypeptide. In some examples, the GOI encodes a protein selected from the group consisting of FVIII protein, FIX protein, alpha-1-antitrypsin, FXIII protein, FVII protein, FX protein, Protein C, Serpin G1, or a functional derivative of any thereof.

[0032] In some examples, the nucleic acid encoding the DNA endonuclease is a deoxyribonucleic acid (DNA) sequence.

[0033] In some examples, the nucleic acid encoding the DNA endonuclease is a ribonucleic acid (RNA) sequence.

[0109] In some examples, (a) and (b) are provided to the cell about 1 to 14 days after (c) is provided to the cell.

[0110] In some examples, the nucleic acid sequence encoding the GOI or functional derivative is inserted into a genomic sequence of the cell.

[0111] In some examples, the insertion is at, within, or near the albumin gene or albumin gene regulatory elements in the genome of the cell.

[0112] In some examples, the insertion is in the first intron of the albumin gene in the genome of the cell.

[0113] 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.

[0114] In some examples, the nucleic acid sequence encoding the GOI or functional derivative is expressed under the control of the endogenous albumin promoter.

[0115] In some examples, the cell is a hepatocyte.

[0116] In some examples, the nucleic acid sequence encoding the GOI, such as a nucleic acid sequence encoding a FVIII protein or functional derivative, is expressed in the liver of the subject.

[0117] In still another aspect, the present disclosure refers to the use of the genetically modified cell of disclosure in the manufacture of a medicament for the treatment of hemophilia B, or hereditary angioedema (HAE) in a subject, wherein the genetically modified cell is to be administered to the subject. In another example, provided herein is a method of treating hemophilia A, hemophilia B, or HAE in a subject. The method includes administering a genetically modified cell as disclosed herein. In some examples, the genetically modified cell is autologous to the subject. In some examples, the method includes (i) obtaining a biological sample from the subject wherein the biological sample includes a hepatocyte cell, wherein the genetically modified cell is prepared from the hepatocyte.

[0118] Each of the examples described herein are capable of being used together, unless excluded either explicitly or clearly from the context of the examples.

[0119] The foregoing summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative examples and features described herein, further examples, objects and features of the disclosure will become fully apparent from the drawings and the detailed description and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0120] An understanding of certain features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative

CLAIMS

WHAT IS CLAIMED IS:

1. A system comprising:

a Cas9 endonuclease or nucleic acid encoding said Cas9 endonuclease;

a guide RNA (gRNA) comprising a spacer sequence from any one of SEQ ID NOs: 22, 21, 28, and 30, or nucleic acid encoding the gRNA; and

a donor template comprising a nucleic acid sequence encoding a protein selected from the group consisting of Factor IX (FIX) protein, alpha-1-antitrypsin, Factor XIII (FXIII) protein, Factor VII (FVII) protein, Factor X (FX) protein, Protein C, and serine protease inhibitor G1 (serpin G1).

2. The system of claim 1, wherein said Cas9 endonuclease recognizes a protospacer adjacent motif (PAM) having the sequence NGG or NNGG, wherein N is any nucleotide.

3. The system of claim 1 or claim 2, wherein the nucleic acid encoding said Cas9 and the nucleic acid encoding said protein is codon optimized for expression in a host cell.

4. The system of any one of claims 1-3, wherein said Cas9 endonuclease or nucleic acid encoding the Cas9 endonuclease is formulated in a liposome or lipid nanoparticle.

5. The system of claim 4, wherein said liposome or lipid nanoparticle also comprises the gRNA.

6. The system of any one of claims 1-5, comprising the Cas9 endonuclease precomplexed with the gRNA, forming a ribonucleoprotein (RNP) complex.

7. The system of any one of claims 1-6, wherein the nucleic acid sequence encodes a FIX protein or G1 protein.

8. A 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, and 30, or nucleic acid encoding the gRNA;

(b) a Cas9 endonuclease or nucleic acid encoding the Cas9 endonuclease; and

(c) a donor template comprising a nucleic acid sequence encoding a protein selected from the group consisting of Factor IX (FIX) protein, alpha-1-antitrypsin, Factor XIII (FXIII) protein, Factor VII (FVII) protein, Factor X (FX) protein, Protein C, and serine protease inhibitor G1 (serpin G1),

wherein the method is not a method for treatment of the human or animal body by therapy.

9. The method of claim 8, wherein said Cas9 endonuclease recognizes a protospacer adjacent motif (PAM) having the sequence NGG or NNGG, wherein N is any nucleotide.

10. The method of claim 8 or claim 9, wherein the nucleic acid encoding said Cas9 and the nucleic acid encoding said protein is codon optimized for expression in the cell.

11. The method of any one of claims 8-10, wherein said Cas9 endonuclease or nucleic acid encoding the Cas9 endonuclease is formulated in a liposome or lipid nanoparticle.

12. The method of claim 11, wherein said liposome or lipid nanoparticle also comprises the gRNA.

13. The method of any one of claims 8-12, comprising providing to the cell the Cas9 endonuclease precomplexed with the gRNA, forming an RNP complex.

14. The method of any one of claims 8-13, wherein the gRNA of (a) and the Cas9 or nucleic acid encoding the Cas9 of (b) are provided to the cell more than 4 days after the donor template of (c) is provided to the cell.

15. The method of any one of claims 8-14, wherein the gRNA of (a) and the Cas9 or nucleic acid encoding the Cas9 of (b) are provided to the cell at least 14 days after (c) is provided to the cell.

16. The method of claim 14 or claim 15, wherein one or more additional doses of the gRNA of (a) and the Cas9 or nucleic acid encoding the Cas9 of (b) are provided to the cell

following the first dose of the gRNA of (a) and the Cas9 or nucleic acid encoding the Cas9 of (b).

17. The method of claim 16, wherein one or more additional doses of the gRNA of (a) and the Cas9 or nucleic acid encoding the Cas9 of (b) are provided to the cell following the first dose of the gRNA of (a) and the Cas9 or nucleic acid encoding the Cas9 of (b) until a target level of targeted integration of the nucleic acid sequence encoding the protein and/or a target level of expression of the nucleic acid sequence encoding the protein is achieved.

18. The method of any one of claims 8-17, wherein the nucleic acid sequence encodes a FIX protein or serpin G1 protein.

19 The method of any one of claims 8-18, wherein the nucleic acid sequence encoding the protein is expressed under the control of the endogenous albumin promoter.

20. The method of any one of claims 8-19, wherein said cell is a hepatocyte.

21. A genetically modified cell in which the genome of the cell is edited by the method of any one of claims 8-20.

22. The system of claim 1 for use in the treatment of a disorder or health condition in a subject, wherein (a), (b) and (c) are to be provided to a cell in the subject.

23. The system for use of claim 22, wherein the nucleic acid sequence encodes a FIX protein or a serpin G1 protein.

24. The system for use of claim 22 or claim 23, wherein said subject is a patient having or diagnosed with a risk of a disorder or health condition selected from the group consisting of hemophilia B, alpha-1-antitrypsin deficiency, FXIII deficiency, FVII deficiency, FX deficiency, Protein C deficiency, and hereditary angioedema (HAE).

25. The system for use of claim 24, wherein the subject is a patient having HAE.

26. The system for use of any one of claims 22-25, wherein (a), (b) and (c) are to be administered via intravenous route.

27. The system for use of any one of claims 22-26, wherein the nucleic acid sequence encoding the protein is expressed in the liver of the subject.

28. Use of the genetically modified cell of claim 21 in the manufacture of a medicament for the treatment of hemophilia B, or hereditary angioedema (HAE) in a subject, wherein the genetically modified cell is to be administered to the subject.

29. The use of claim 28, wherein said genetically modified cell is autologous to the subject.

30. The use of claim 28 or 29, wherein the genetically modified cell is prepared from a biological sample which is to be obtained from the subject, wherein the biological sample comprises a hepatocyte cell.