SUMMARY

[0018] The present disclosure refers to subject matter as defined in the attached set of claims. Provided herein are genome editing systems, ribonucleoprotein (RNP) complexes, guide RNAs, Cpf1 proteins, including modified Cpf1 proteins (Cpf1 variants), and CRISPR-mediated methods for altering the promoter region of one or more γ–globin genes (e.g., *HBG1*, *HBG2*, or *HBG1* and *HBG2*) and increasing expression of fetal hemoglobin (HbF). In certain embodiments, an RNP complex may include a guide RNA (gRNA) complexed to a wild-type Cpf1 or modified Cpf1 RNA-guided nuclease (modified Cpf1 protein). In certain embodiments, a gRNA may comprise a sequence set forth in **Table 13**, **Table 18**, or **Table 19**. In certain embodiments, a gRNA may comprise a gRNA targeting domain. In certain embodiments, a gRNA targeting domain may comprise a sequence selected from the group consisting of SEQ ID NOs:1002, 1254, 1258, 1260, 1262, and 1264. In certain embodiments, a gRNA may comprise a gRNA sequence set forth in **Table 19**. In certain embodiments, a gRNA may comprise a sequence selected from the group consisting of SEQ ID NOs:1022, 1023, 1041-1105. In certain embodiments, the RNP complex may comprise an RNP complex set forth in **Table 21**. For example, an RNP complex may include a gRNA comprising the sequence set forth in SEQ ID NO:1051 and a modified Cpf1 protein encoded by the sequence set forth in SEQ ID NO:1097 (RNP32, **Table 21**).

[0019] The inventors have discovered herein that delivery of an RNP complex including a gRNA complexed to a modified Cpf1 protein may result in increased editing of a target nucleic acid. In certain embodiments, the modified Cpf1 protein may contain one or more modifications. In certain embodiments, the one or more modifications may include, without limitation, one or more mutations in a wild-type Cpf1 amino acid sequence, one or more mutations in a wild-type Cpf1 nucleic acid sequence, one or more nuclear localization signals (NLS), one or more purification tags (e.g., His tag), or a combination thereof. In certain embodiments, a modified Cpf1 may be encoded by a sequence set forth in SEQ ID NOs:1000, 1001, 1008-1018, 1032, 1035-39, 1094-1097, 1107-09 (Cpf1 polypeptide sequences) or SEQ ID NOs:1019-1021, 1110-17 (Cpf1 polynucleotide sequences). In certain embodiments, the gRNA may be a modified or unmodified gRNA. In certain embodiments, the gRNA may comprise a sequence set forth in **Table 13**, **Table 18**, or **Table 19**. In certain embodiments, the RNP complex may comprise an RNP complex set forth in **Table 21**. For example, the RNP complex may include a gRNA comprising the sequence set forth in SEQ ID NO:1051 and a modified Cpf1 protein encoded by the sequence set forth in SEQ ID NO:1097 (RNP32, **Table 21**). In certain embodiments, an RNP complex comprising a modified Cpf1 protein may increase editing of a target nucleic acid. In certain embodiments, an RNP complex comprising a modified Cpf1 protein may increase editing resulting in an increase of productive indels. In various embodiments, an increase in editing of the target nucleic acid may be assessed by any means known to skilled artisans, such as, but not limited to, PCR amplification of

CLAIMS

1. A ribonucleoprotein (RNP) complex comprising :

a Cpfl variant comprising one or more modifications selected from the group consisting of one or more mutations in a wild-type Cpfl amino acid sequence, one or more nuclear localization signals, one or more purification tags, and a combination thereof and

a gRNA comprising:

a 5' end and a 3' end,

a DNA extension at the 5' end, wherein the DNA extension comprises a sequence selected from the group consisting of SEQ ID NOs: 1235-1250,

a 2'-O-methyl modification and a phosphorothioate modification at the 3' end,

a targeting domain that is complementary to a target site in a promoter of a gamma-globin (HBG) gene,

wherein the target site comprises nucleotides located between Chr 11 (NC_000011.10) 5,249,955 – 5,249,987; Chr 11 (NC_000011.10) 5,254,879 – 5,254,909; or a combination thereof, and

an RNA segment capable of associating with the Cpfl variant.

2. The RNP complex of claim 1, wherein the targeting domain comprises a sequence selected from the group consisting of SEQ ID NOs: 1002, 1004, 1139, 1141, 1143, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, 1195, 1197, 1199, 1201, 1203, 1205, 1207, 1209, 1211, 1213, 1215, 1217, 1219, 1221, 1223, 1225, 1227, 1229, 1254, 1256, 1258, 1260, 1262, and 1264.

3. The RNP complex of claim 2, wherein the Cpfl variant comprises a sequence selected from the group consisting of SEQ ID NOs: 1000, 1001, 1008-1015, and 1035-39.

4. A method of altering a promoter of a gamma-globin (*HBG*) gene in a cell comprising: contacting the cell with an RNP complex comprising:

a Cpfl variant comprising one or more modifications selected from the group consisting of one or more mutations in a wild-type Cpfl amino acid sequence, one or more nuclear localization signals, one or more purification tags, and a combination thereof and

a gRNA comprising:

a 5’ end and a 3’ end,

a DNA extension at the 5’ end, wherein the DNA extension comprises a sequence selected from the group consisting of SEQ ID NOs: 1235-1250,

a 2’-O-methyl modification and a phosphorothioate modification at the 3’ end,

a targeting domain that is complementary to a target site in the promoter of the *HBG* gene,

wherein the target site comprises nucleotides located between Chr 11 (NC_000011.10) 5,249,955 – 5,249,987; Chr 11 (NC_000011.10) 5,254,879 – 5,254,909; or a combination thereof, and

an RNA segment capable of associating with the Cpfl variant.

5. The method of claim 4, wherein the targeting domain comprises a sequence selected from the group consisting of SEQ ID NOs: 1002, 1004, 1139, 1141, 1143, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, 1195, 1197, 1199, 1201, 1203, 1205, 1207, 1209, 1211, 1213, 1215, 1217, 1219, 1221, 1223, 1225, 1227, 1229, 1254, 1256, 1258, 1260, 1262, and 1264.

6. The method of claim 5, wherein the Cpfl variant comprises a sequence selected from the group consisting of SEQ ID NOs: 1000, 1001, 1008-1015, and 1035-39.

7. The method of claim 5, wherein the cell is a CD34+ cell or a hematopoietic stem cell.

8. The method of claim 5, wherein the RNP complex is delivered to the cell using electroporation.

9. An *ex vivo* method of increasing the level of fetal hemoglobin (HbF) in a human cell by genome editing using an RNP complex comprising:

a Cpfl variant comprising one or more modifications selected from the group consisting of one or more mutations in a wild-type Cpfl amino acid sequence, one or more nuclear localization signals, one or more purification tags, and a combination thereof and

a gRNA comprising:

a 5’ end and a 3’ end,

a DNA extension at the 5' end, wherein the DNA extension comprises a sequence selected from the group consisting of SEQ ID NOs: 1235-1250,

a 2'-O-methyl modification and a phosphorothioate modification at the 3' end,

a targeting domain that is complementary to a target site in a promoter of a gamma-globin (*HBG*) gene,

wherein the target site comprises nucleotides located between Chr 11 (NC_000011.10) 5,249,955 – 5,249,987; Chr 11 (NC_000011.10) 5,254,879 – 5,254,909; or a combination thereof, and

an RNA segment capable of associating with the Cpfl variant to affect an alteration in the promoter of the *HBG* gene, thereby to increase expression of HbF.

10. The method of claim 9, wherein the targeting domain comprises a sequence selected from the group consisting of SEQ ID NOs: 1002, 1004, 1139, 1141, 1143, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, 1195, 1197, 1199, 1201, 1203, 1205, 1207, 1209, 1211, 1213, 1215, 1217, 1219, 1221, 1223, 1225, 1227, 1229, 1254, 1256, 1258, 1260, 1262, and 1264.

11. The method of claim 10, wherein the Cpf1 variant comprises a sequence selected from the group consisting of SEQ ID NOs: 1000, 1001, 1008-1015, and 1035-39.

12. The method of claim 10, wherein the cell is a CD34+ cell or a hematopoietic stem cell.

13. The method of claim 10, wherein the RNP complex is delivered to the cell using electroporation.

14. A ribonucleoprotein (RNP) complex comprising:

a Cpfl variant comprising one or more modifications selected from the group consisting of one or more mutations in a wild-type Cpf1 amino acid sequence, one or more nuclear localization signals, one or more purification tags, and a combination thereof,

wherein the Cpf1 variant comprises a sequence selected from the group consisting of SEQ ID NOs: 1000, 1001, 1008-1015, and 1035-39, and

a gRNA comprising:

a 5' end and a 3' end,

a DNA extension at the 5' end,

wherein the DNA extension comprises a sequence selected from the group consisting of SEQ ID NOs: 1235-1250;

a 2'-O-methyl modification and a phosphorothioate modification at the 3' end,

a targeting domain that is complementary to a target site in a promoter of a gamma-globin (*HBG*) gene,

wherein the targeting domain comprises a sequence selected from the group consisting of SEQ ID NOs: 1002, 1004, 1139, 1141, 1143, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, 1195, 1197, 1199, 1201, 1203, 1205, 1207, 1209, 1211, 1213, 1215, 1217, 1219, 1221, 1223, 1225, 1227, 1229, 1254, 1256, 1258, 1260, 1262, and 1264; and

an RNA segment capable of associating with the Cpf1 variant.

15. A method of altering a promoter of a gamma-globin (*HBG*) gene in a cell comprising: contacting the cell with an RNP complex comprising:

a Cpfl variant comprising one or more modifications selected from the group consisting of one or more mutations in a wild-type Cpfl amino acid sequence, one or more nuclear localization signals, one or more purification tags, and a combination thereof,

wherein the Cpfl variant comprises a sequence selected from the group consisting of SEQ ID NOs: 1000, 1001, 1008-1015, and 1035-39, and

a gRNA comprising:

a 5' end and a 3' end,

a DNA extension at the 5' end,

wherein the DNA extension comprises a sequence selected from the group consisting of SEQ ID NOs: 1235-1250,

a 2'-O-methyl modification and a phosphorothioate modification at the 3' end,

a targeting domain that is complementary to a target site in the promoter of the *HBG* gene,

wherein the targeting domain comprises a sequence selected from the group consisting of SEQ ID NOs: 1002, 1004, 1139, 1141, 1143, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1167, 1169, 1171,

1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, 1195, 1197, 1199, 1201, 1203, 1205, 1207, 1209, 1211, 1213, 1215, 1217, 1219, 1221, 1223, 1225, 1227, 1229, 1254, 1256, 1258, 1260, 1262, and 1264, and

an RNA segment capable of associating with the Cpfl variant.

16. The method of claim 15, wherein the cell is a CD34+ cell or a hematopoietic stem cell.

17. The method of claim 15, wherein the RNP complex is delivered to the cell using electroporation.

18. An *ex vivo* method of increasing the level of fetal hemoglobin (HbF) in a human cell by genome editing using an RNP complex comprising:

a Cpfl variant comprising one or more modifications selected from the group consisting of one or more mutations in a wild-type Cpf1 amino acid sequence, one or more nuclear localization signals, one or more purification tags, and a combination thereof,

wherein the Cpfl variant comprises a sequence selected from the group consisting of SEQ ID NOs: 1000, 1001, 1008-1015, and 1035-39, and

a gRNA comprising:

a 5' end and a 3' end,

a DNA extension at the 5' end,

wherein the DNA extension comprises a sequence selected from the group consisting of SEQ ID NOs: 1235-1250,

a 2'-O-methyl modification and a phosphorothioate modification at the 3' end,

a targeting domain that is complementary to a target site in a promoter of a gamma-globin (*HBG*) gene,

wherein the targeting domain comprises a sequence selected from the group consisting of SEQ ID NOs: 1002, 1004, 1139, 1141, 1143, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, 1195, 1197, 1199, 1201, 1203, 1205, 1207, 1209, 1211, 1213, 1215, 1217, 1219, 1221, 1223, 1225, 1227, 1229, 1254, 1256, 1258, 1260, 1262, and 1264,

and

an RNA segment capable of associating with the Cpfl variant to affect an alteration in the promoter of the *HBG* gene, thereby to increase expression of HbF.

19. The method of claim 18, wherein the cell is a CD34+ cell or a hematopoietic stem cell.

20. The method of claim 18, wherein the RNP complex is delivered to the cell using electroporation.