decade. At that time, they require chelation therapy (in addition to continued transfusions) to prevent complications of iron overload. Iron overload may manifest as growth delay or delay of sexual maturation. In adulthood, inadequate chelation therapy may lead to cardiomyopathy, cardiac arrhythmias, hepatic fibrosis and/or cirrhosis, diabetes, thyroid and parathyroid abnormalities, thrombosis, and osteoporosis. Frequent transfusions also put subjects at risk for infection with HIV, hepatitis B and hepatitis C.

[0015] β-Thal intermedia subjects generally present between the ages of 2-6 years. They do not generally require blood transfusions. However, bone abnormalities occur due to chronic hypertrophy of the erythroid lineage to compensate for chronic anemia. Subjects may have fractures of the long bones due to osteoporosis. Extramedullary erythropoiesis is common and leads to enlargement of the spleen, liver, and lymph nodes. It may also cause spinal cord compression and neurologic problems. Subjects also suffer from lower extremity ulcers and are at increased risk for thrombotic events, including stroke, pulmonary embolism, and deep vein thrombosis. Treatment of β-Thal intermedia includes splenectomy, folic acid supplementation, hydroxyurea therapy, and radiotherapy for extramedullary masses. Chelation therapy is used in subjects who develop iron overload.

[0016] Life expectancy is often diminished in β-Thal patients. Subjects with β-Thal major who do not receive transfusion therapy generally die in their second or third decade. Subjects with β-Thal major who receive regular transfusions and adequate chelation therapy can live into their fifth decade and beyond. Cardiac failure secondary to iron toxicity is the leading cause of death in β-Thal major subjects due to iron toxicity.

[0017] A variety of new treatments are currently in development for SCD and β-Thal. Delivery of an anti-sickling *HBB* gene via gene therapy is currently being investigated in clinical trials. However, the long-term efficacy and safety of this approach is unknown. Transplantation with hematopoietic stem cells (HSCs) from an HLA-matched allogeneic stem cell donor has been demonstrated to cure SCD and β-Thal, but this procedure involves risks including those associated with ablation therapy, which is required to prepare the subject for transplant, increases risk of life-threatening opportunistic infections, and risk of graft vs. host disease after transplantation. In addition, matched allogeneic donors often cannot be identified. Thus, there is a need for improved methods of managing these and other hemoglobinopathies.

SUMMARY

[0018] Described herein in certain examples are first populations of modified cells comprising a plurality of modified CD34+ or hematopoietic stem cells with one or more indels in an *HBG* gene promoter. In certain of these examples, the plurality of modified cells include an indel in a CCAAT box target region.

[0019] In one aspect, the present disclosure refers to a first population of modified cells comprising a plurality of modified CD34+ or hematopoietic stem cells, one or more of the plurality of modified cells having an indel in an HBG gene promoter, the plurality of modified cells as a whole comprising the following deletions: a HBG1/2 c.-104 to -121 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-104 to -121 deletion making up 1% to 15.5% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-110 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c. -110 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-112 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-112 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-113 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-113 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-111 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-111 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-111 to -117 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-111 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-102 to -114 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-102 to -114 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-114 to -118 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-114 to -118 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-112 to -116 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-112 to -116 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; and a HBG1/2 c.-113 to -117 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-113 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole. In another aspect, the present disclosure refers to a first population of modified cells comprising a plurality of modified CD34+ or hematopoietic stem cells, one or more of the plurality of modified cells including an indel in an HBG gene promoter, and 60% or more of the indels in the plurality of modified cells as a whole are deletions of at least 4 base pairs the plurality of modified cells as a whole comprising the following deletions: a HBG1/2 c.-104 to -121 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-104 to -121 deletion making up 1% to 15.5% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-110 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c. -110 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-112 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-112 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-113 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-113 to -115 deletion making up

1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-111 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-111 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-111 to -117 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-111 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-102 to -114 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-102 to -114 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-114 to -118 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-114 to -118 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-112 to -116 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-112 to -116 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; and a HBG1/2 c.-113 to -117 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-113 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole. In yet another aspect, the present disclosure refers to a first population of modified cells comprising: a plurality of modified CD34+ or hematopoietic stem cells, one or more of the plurality of modified cells including an indel in an HBG gene promoter, and 60% or more of the indels in the plurality of modified cells as a whole are deletions of at least 4 base pairs; the indels generated by delivering a first RNP complex including a first gRNA and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a first population of unmodified cells comprising a plurality of unmodified CD34+ or hematopoietic stem cells, the first gRNA including a first gRNA targeting domain, the plurality of modified cells as a whole comprising the following deletions: a HBG1/2 c.-104 to -121 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-104 to -121 deletion making up 1% to 15.5% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-110 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c. -110 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-112 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-112 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-113 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-113 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-111 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-111 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-111 to -117 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-111 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-102 to -114 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-102 to -114 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-114 to -118 deletion in a HBG1

promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-114 to -118 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-112 to -116 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-112 to -116 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; and a HBG1/2 c.-113 to -117 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-113 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole. In another aspect, the present disclosure refers to an *ex vivo* or *in vitro* method of inducing expression of fetal hemoglobin (HbF) in a first population of modified cells comprising a plurality of modified CD34+ or hematopoietic stem cells, the method comprising: delivering a first RNP complex including a first guide RNA (gRNA) and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a first population of unmodified cells comprising a plurality of unmodified CD34+ or hematopoietic stem cells to generate indels, the first gRNA including a first gRNA targeting domain, each modified CD34+ or hematopoietic stem cell comprising an indel in an HBG gene promoter, 60% or more of the indels in the plurality of modified cells as a whole are deletions of at least 4 base pairs; the first population of modified cells comprising higher HbF levels than the first population of unmodified cells, the plurality of modified cells as a whole comprising the following deletions: a HBG1/2 c.-104 to -121 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-104 to -121 deletion making up 1% to 15.5% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-110 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c. -110 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-112 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-112 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-113 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-113 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-111 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-111 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-111 to -117 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-111 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-102 to -114 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-102 to -114 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-114 to -118 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-114 to -118 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-112 to -116 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-112 to -116 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; and a HBG1/2 c.-113 to -117 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-113 to -117 deletion making up 1% to 6%

of the indels in the plurality of modified cells as a whole. In a further aspect, the present disclosure refers to a method of decreasing sickling in a first population of red blood cells (RBCs) cultured from a first population of modified cells comprising a plurality of modified CD34+ cells from a subject having sickle cell disease, the method comprising: a) delivering a first RNP complex including a first gRNA and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a first population of unmodified cells comprising a plurality of unmodified CD34+ cells obtained from the subject to generate indels, the first gRNA including a first gRNA targeting domain, each modified CD34+ cell comprising an indel in an HBG gene promoter, 60% or more of the indels in the plurality of modified CD34+ cells as a whole are deletions of at least 4 base pairs; and b) culturing the first population of RBCs from the first population of cells comprising the plurality of modified CD34+ cells, the first population of RBCs exhibiting significantly decreased sickling upon deoxygenation than a second population of RBCs comprising a plurality of RBCs cultured from the first population of unmodified cells , the plurality of modified cells as a whole comprising the following deletions: a HBG1/2 c.-104 to -121 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-104 to -121 deletion making up 1% to 15.5% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-110 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c. -110 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-112 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-112 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-113 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-113 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-111 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-111 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-111 to -117 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-111 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-102 to -114 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-102 to -114 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-114 to -118 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-114 to -118 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-112 to -116 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-112 to -116 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; and a HBG1/2 c.-113 to -117 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-113 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole. In yet another aspect, the present disclosure refers to the use of a first population of modified cells comprising a plurality of modified CD34+ or hematopoietic stem cells in the manufacture of a medicament for alleviating one or more symptoms of

sickle cell disease in a subject in need thereof, wherein the manufacture of the medicament comprises delivering a first RNP complex including a first gRNA and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a first population of unmodified cells comprising a plurality of unmodified CD34+ or hematopoietic stem cells to generate indels, each modified CD34+ or hematopoietic stem comprising an indel in an HBG gene promoter, 60% or more of the indels in a plurality of the modified CD34+ or hematopoietic stem cells as a whole are deletions of at least 4 base pairs, the plurality of modified cells as a whole comprising the following deletions: a HBG1/2 c.-104 to -121 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-104 to -121 deletion making up 1% to 15.5% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-110 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c. -110 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-112 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-112 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-113 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-113 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-111 to -115 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-111 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-111 to -117 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-111 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-102 to -114 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-102 to -114 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-114 to -118 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-114 to -118 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; a HBG1/2 c.-112 to -116 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-112 to -116 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; and a HBG1/2 c.-113 to -117 deletion in a HBG1 promoter, HBG2 promoter, or a combination thereof, the HBG1/2 c.-113 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole. In certain examplesof the first populations of modified cells described herein, one or more of the cells in the plurality of modified cells include a HBG1/2 c.-104 to -121 deletion in a *HBG1* promoter, an *HBG2* promoter, or both. In certain of these examples, HBG1/2 c.-104 to -121 deletions make up 1% or more, 1.5% or more, 2% or more, 2.5% or more. 3% or more, 3.5% or more, 4% or more, 4.5% or more, 5% or more, 5.5% or more, 6% or more, 6.5% or more, 7% or more, 7.5% or more, 8% or more, 8.5% or more, 9% or more, 9.5% or more, 10% or more, 10.5% or more, 11% or more, 11.5% or more, 12% or more, 12.5% or more, 13% or more, 13.5% or more, 14% or more, 14.5% or more, 15% or more, or 15.5% or more of the indels in the

plurality of modified cells as a whole. In certain of these examples, HBG1/2 c.-104 to -121 deletions make up less than 25% of the indels in the plurality of modified cells as a whole.

[0020] In certain examplesof the first populations of modified cells provided herein, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, or 95% or more of the indels in the plurality of modified cells as a whole are deletions of at least 4 base pairs.

[0021] In certain examplesof the first populations of modified cells provided herein, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, or 65% or more of the indels in the plurality of modified cells as a whole are deletions of at least 4 base pairs introduced by a repair mechanism other than microhomology-mediated end joining (MMEJ) repair.

[0022] In certain examplesof the first populations of modified cells provided herein, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, or 65% or more of the indels in the plurality of modified cells as a whole are deletions of at least 4 base pairs introduced by non-homologous end joining (NHEJ) repair, e.g., canonical NHEJ repair.

[0023] In certain examplesof the first populations of modified cells provided herein, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, or 92% or more of the indels in the plurality of modified cells as a whole are deletions of 1 to 25 base pairs.

[0024] In certain examplesof the first populations of modified cells provided herein, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, or 85% or more of the indels in the plurality of modified cells as a whole are deletions of 3 to 25 base pairs.

[0025] In certain examplesof the first populations of modified cells provided herein, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, or 80% or more of the indels in the plurality of modified cells as a whole are deletions of 4 to 25 base pairs.

[0026] In certain examplesof the first populations of modified cells provided herein, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, or 72% or more of the indels in the plurality of modified cells as a whole are deletions of 5 to 25 base pairs.

[0027] In certain examplesof the first populations of modified cells provided herein, the modified cells are produced by delivering a first RNP complex including a first gRNA comprising a first gRNA targeting domain and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a first population of unmodified cells comprising a plurality of unmodified CD34+ or hematopoietic stem cells to generate indels. In certain of these examples, the first RNP complex is delivered to the first population of unmodified cells by electroporation. In certain examples, the first population of unmodified cells is from a subject having sickle cell disease. In certain examples, the first gRNA includes a 5' end and a 3' end, with a DNA extension at the 5' end and a 2'-O-methyl-3'-phosphorothioate modification at the 3' end. In certain of these examples, the DNA extension at the 5' end comprises a sequence set forth in any of SEQ ID NOs:1235-1250. In certain examples, the first gRNA targeting domain comprises the sequence set forth in SEQ ID NO:1254. In certain examples, the first gRNA comprises the sequence set forth in SEQ ID NO:1051. In certain examples, the modified Cpf1 RNA-guided nuclease comprises the sequence set forth in SEQ ID NO:1097. In certain examples, the first population of modified cells has higher fetal hemoglobin (HbF) levels than the first population of unmodified cells.

[0028] In certain examplesof the first populations of modified cells provided herein, one or more of the cells in the plurality of modified cells include (a) a HBG1/2 c.-104 to -121 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (b) a HBG1/2 c.-110 to -115 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (c) a HBG1/2 c.-112 to -115 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (d) a HBG1/2 c.-113 to -115 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (e) a HBG1/2 c.-111 to -115 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (f) a HBG1/2 c.-111 to -117 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (g) a HBG1/2 c.-102 to -114 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (h) a HBG1/2 c.-114 to -118 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (i) a HBG1/2 c.-112 to -116 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; or (j) a HBG1/2 c.-113 to -117 deletion in a *HBG1* promoter, an *HBG2* promoter, or both.

[0029] In certain examplesof the first populations of modified cells provided herein, the plurality of modified cells as a whole includes (a) a HBG1/2 c.-104 to -121 deletion in a *HBG1* promoter, an *HBG2* promoter, or both and (b) a HBG1/2 c.-110 to -115 deletion in a *HBG1* promoter, an *HBG2* promoter, or both. In certain examples, HBG1/2 c.-104 to -121 deletions make up 1% or more, 1.5% or more, 2% or more, 2.5% or more. 3% or more, 3.5% or more, 4% or more, 4.5% or more, 5% or more, 5.5% or more, 6% or more, 6.5% or more, 7% or more, 7.5% or more, 8% or more, 8.5% or more, 9% or more, 9.5% or more, 10% or more, 10.5% or more, 11% or more, 11.5% or more, 12% or more, 12.5% or more, 13% or more, 13.5% or more, 14% or more, 14.5% or more, or 15% or more of the indels in the plurality of modified cells as a whole. In certain examples, HBG1/2 c.-104 to -121 deletions make up 1% to 15.5% of the indels in the plurality of modified cells as a whole. In

certain examples, HBG1/2 c.-110 to -115 deletions make up 0.5% or more, 1% or more, 1.5% or more, 2% or more, 2.5% or more. 3% or more, 3.5% or more, 4% or more, 4.5% or more, 5% or more, or 5.5% or more of the indels in the plurality of modified cells as a whole. In certain examples, HBG1/2 c.-110 to -115 deletions make up 0.5% to 6% of the indels in the plurality of modified cells as a whole.

[0030] In certain examplesof the first populations of modified cells provided herein, the plurality of modified cells as a whole include (a) a HBG1/2 c.-104 to -121 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (b) a HBG1/2 c.-110 to -115 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (c) a HBG1/2 c.-112 to -115 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (d) a HBG1/2 c.-113 to -115 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (e) a HBG1/2 c.-111 to -115 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (f) a HBG1/2 c.-111 to -117 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (g) a HBG1/2 c.-102 to -114 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (h) a HBG1/2 c.-114 to -118 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; (i) a HBG1/2 c.-112 to -116 deletion in a *HBG1* promoter, an *HBG2* promoter, or both; and (j) a HBG1/2 c.-113 to -117 deletion in a *HBG1* promoter, an *HBG2* promoter, or both. In certain examples, the HBG1/2 c.-104 to -121 deletions make up 1% or more, 1.5% or more, 2% or more, 2.5% or more. 3% or more, 3.5% or more, 4% or more, 4.5% or more, 5% or more, 5.5% or more, 6% or more, 6.5% or more, 7% or more, 7.5% or more, 8% or more, 8.5% or more, 9% or more, 9.5% or more, 10% or more, 10.5% or more, 11% or more, 11.5% or more, 12% or more, 12.5% or more, 13% or more, 13.5% or more, 14% or more, 14.5% or more, or 15% or more of the indels in the plurality of modified cells as a whole. In certain examples, the HBG1/2 c.-104 to -121 deletions make up 1% to 15.5% of the indels in the plurality of modified cells as a whole. In certain examples, the HBG1/2 c.-110 to -115 deletions make up 0.5% or more, 1% or more, 1.5% or more, 2% or more, 2.5% or more. 3% or more, 3.5% or more, 4% or more, 4.5% or more, 5% or more, or 5.5% or more of the indels in the plurality of modified cells as a whole. In certain examples, HBG1/2 c.-110 to -115 deletions make up 0.5% to 6% of the indels in the plurality of modified cells as a whole. In certain examples, the HBG1/2 c.-112 to -115 deletions make up 0.5% or more, 1% or more, 1.5% or more, 2% or more, 2.5% or more. 3% or more, 3.5% or more, 4% or more, 4.5% or more, 5% or more, or 5.5% or more of the indels in the plurality of modified cells as a whole. In certain examples, HBG1/2 c.-112 to -115 deletions make up 0.5% to 6% of the indels in the plurality of modified cells as a whole. In certain examples, the HBG1/2 c.-113 to -115 deletions make up 0.5% or more, 1% or more, 1.5% or more, 2% or more, 2.5% or more. 3% or more, 3.5% or more, 4% or more, 4.5% or more, 5% or more, or 5.5% or more of the indels in the plurality of modified cells as a whole. In certain examples, HBG1/2 c.-113 to -115 deletions make up 0.5% to 6% of the indels in the plurality of modified cells as a whole. In certain examples, the HBG1/2 c.-111 to -115 deletions make up 0.5% or more, 1% or more, 1.5%

or more, 2% or more, 2.5% or more. 3% or more, 3.5% or more, 4% or more, 4.5% or more, 5% or more, or 5.5% or more of the indels in the plurality of modified cells as a whole. In certain examples, HBG1/2 c.-111 to -115 deletions make up 0.5% to 6% of the indels in the plurality of modified cells as a whole. In certain examples, the HBG1/2 c.-111 to -117 deletions make up 0.5% or more, 1% or more, 1.5% or more, 2% or more, 2.5% or more. 3% or more, 3.5% or more, 4% or more, 4.5% or more, 5% or more, or 5.5% or more of the indels in the plurality of modified cells as a whole. In certain examples, HBG1/2 c.-111 to -117 deletions make up 0.5% to 6% of the indels in the plurality of modified cells as a whole. In certain examples, the HBG1/2 c.-102 to -114 deletions make up 0.5% or more, 1% or more, 1.5% or more, 2% or more, 2.5% or more. 3% or more, 3.5% or more, 4% or more, 4.5% or more, 5% or more, or 5.5% or more of the indels in the plurality of modified cells as a whole. In certain examples, HBG1/2 c.-102 to -114 deletions make up 0.5% to 6% of the indels in the plurality of modified cells as a whole. In certain examples, the HBG1/2 c.-114 to -118 deletions make up 0.5% or more, 1% or more, 1.5% or more, 2% or more, 2.5% or more. 3% or more, 3.5% or more, 4% or more, 4.5% or more, 5% or more, or 5.5% or more of the indels in the plurality of modified cells as a whole. In certain examples, HBG1/2 c.-114 to -118 deletions make up 0.5% to 6% of the indels in the plurality of modified cells as a whole. In certain examples, the HBG1/2 c.-112 to -116 deletions make up 0.5% or more, 1% or more, 1.5% or more, 2% or more, 2.5% or more. 3% or more, 3.5% or more, 4% or more, 4.5% or more, 5% or more, or 5.5% or more of the indels in the plurality of modified cells as a whole. In certain examples, HBG1/2 c.-112 to -116 deletions make up 0.5% to 6% of the indels in the plurality of modified cells as a whole. In certain examples, the HBG1/2 c.-113 to -117 deletions make up 0.5% or more, 1% or more, 1.5% or more, 2% or more, 2.5% or more. 3% or more, 3.5% or more, 4% or more, 4.5% or more, 5% or more, or 5.5% or more of the indels in the plurality of modified cells as a whole. In certain examples, HBG1/2 c.-113 to -117 deletions make up 0.5% to 6% of the indels in the plurality of modified cells as a whole.

[0031] In certain examplesof the first populations of modified cells provided herein, the plurality of modified cells as a whole includes the 108 deletions present in all 14 samples in Table 25.

[0032] In certain examplesof the first populations of modified cells provided herein, the plurality of modified cells as a whole includes the indels identified as having an “Ave % in Indel” of 0.1% or more in Table 25. In certain of these examples, the plurality of modified cells as a whole includes all of the indels in Table 25.

[0033] In certain examplesof the first populations of modified cells provided herein, the plurality of modified cells as a whole include at least 10% more deletions of at least 4 base pairs than a second population of modified cells comprising a plurality of modified CD34+ or hematopoietic stem cells comprising a plurality of modified CD34+ or hematopoietic stem cells with one or more indels in an *HBG* gene promoter, where the indels of the second population of modified cells are generated by

delivering a second RNP complex including a second gRNA having a gRNA targeting domain comprising SEQ ID NO:339 and a Cas9 RNA-guided nuclease to a second population of unmodified cells comprising a plurality of unmodified CD34+ or hematopoietic stem cells. In certain of these examples, the second RNP complex is delivered to the second population of unmodified cells by electroporation. In certain examples, the second population of unmodified cells is from a subject having sickle cell disease. In certain examples, the first population of modified cells has higher HbF levels than the second population of modified cells. In certain of these examples, the plurality of modified cells in the second population include an indel in a CCAAT box target region.

[0034] Described herein in certain examplesare methods of inducing expression of HbF in a first population of modified cells comprising a plurality of modified CD34+ or hematopoietic stem cells with one or more indels in an *HBG* gene promoter, the method comprising delivering a first RNP complex including a first gRNA comprising a first gRNA targeting domain and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a first population of unmodified cells comprising a plurality of unmodified CD34+ or hematopoietic stem cells to generate indels. In certain examples, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, or 95% or more of the resultant indels in the plurality of modified cells as a whole are deletions of at least 4 base pairs. In certain examples, the first population of modified cells exhibits increased HbF levels versus the first population of unmodified cells. In certain examples, the first RNP complex is delivered to the first population of unmodified cells by electroporation.

[0035] Described herein in certain examplesare methods of decreasing sickling in a first population of red blood cells (RBCs) cultured from a first population of modified cells comprising a plurality of modified CD34+ cells with one or more indels in an *HBG* gene promoter, the method comprising delivering a first RNP complex including a first gRNA comprising a first gRNA targeting domain and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a first population of unmodified cells comprising a plurality of unmodified CD34+ cells to generate indels, then culturing the first population of RBCs from the first population of modified cells. In certain examples, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, or 95% or more of the resultant indels in the plurality of modified cells as a whole are deletions of at least 4 base pairs. In certain examples, the first population of RBCs exhibits significantly decreased sickling upon deoxygenation versus a second population of RBCs cultured from the first population of unmodified cells. In certain examples, the first population of RBCs sickle at a significantly lower oxygen tension, for example as measured by relative oxygen pressure, than the second population of RBCs. In certain examples, the first population of RBCs has a significantly higher minimum elongation index upon deoxygenation than the second population of RBCs. In certain examples, the first population of RBCs has a significantly higher velocity upon

deoxygenation than the second population of RBCs. In certain examples, the first population of RBCs has higher HbF levels than the second population of RBCs.

[0036] Described herein in certain examplesare methods of alleviating one or more symptoms of sickle cell disease in a subject in need thereof comprising delivering a first RNP complex including a first gRNA comprising a first targeting domain and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a first population of unmodified cells comprising a plurality of unmodified CD34+ or hematopoietic stem cells to generate a first population of modified cells comprising a plurality of modified CD34+ or hematopoietic stem cells comprising one or more indels in an HBG gene promoter, and then administering the resultant first population of modified cells to the subject to alleviate one or more symptoms of sickle cell disease. In certain examples, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, or 95% or more of the resultant indels in the resultant plurality of modified CD34+ or hematopoietic stem cells as a whole are deletions of at least 4 base pairs. In certain examples, the methods further comprise detecting a population of modified erythroid progeny cells comprising a plurality of modified erythroid progeny cells cultured from the first population of modified cells at about 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 10 weeks, 12 weeks. 16 weeks, 20 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 8 months, 12 months, 1 year, 2 years, 3 years, 4 years, 5 years, or more than 5 years after administration. In certain examples, the cultured cells may include bone marrow (BM)-engrafted CD34+ hematopoietic stem cells or blood cells derived therefrom, e.g., myeloid progenitor or differentiated myeloid cells, e.g., erythrocytes, mast cells, myoblasts; or lymphoid progenitors or differentiated lymphoid cells, e.g., T- or B-lymphocytes or NK cells. In certain examples, the method results in long-term engraftment of a plurality of HSC clones in bone marrow, e.g., at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 10 weeks, 12 weeks. 16 weeks, 20 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 8 months, 12 months, 1 year, 2 years, 3 years, 4 years, 5 years, or more than 5 years after administration. In certain examplesthe method results in long-term expression of at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% of total hemoglobin as compared to a healthy subject. In certain examples, the method results in a reconstitution of all hematopoietic cell lineages, e.g., without any differentiation bias, e.g., without an erythroid lineage differentiation bias.

[0037] Described herein in certain examplesis a population of cells comprising a plurality of red blood cells (RBCs) cultured from a plurality of modified CD34+ cells from a subject having sickle cell disease, each modified cell comprising an indel in an HBG gene promoter. In certain examples, the plurality of modified cells as a whole includes the 108 deletions present in all 14 samples in Table 25. In certain examples, the population of cells is generated by delivering an RNP complex including a gRNA comprising a first targeting domain and a Cpf1 RNA-guided

nuclease or a modified Cpf1 RNA-guided nuclease to a plurality of unmodified CD34+ cells from a subject having sickle cell disease to generate the indels; and culturing the RBCs from the plurality of modified CD34+ cells.

[0038] Described herein in certain examplesis a population of cells comprising a plurality of red blood cells (RBCs) cultured from a plurality of modified CD34+ cells from a subject having sickle cell disease, each modified cell comprising an indel in an HBG gene promoter. In certain examples, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, or 95% or more of the indels in the HBG gene promoter are deletions of at least 4 base pairs. In certain examples, the population of cells is generated by delivering an RNP complex including a gRNA comprising a first targeting domain and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a plurality of unmodified CD34+ cells from a subject having sickle cell disease to generate the indels; and culturing the RBCs from the plurality of modified CD34+ cells.

[0039] In certain examplesof the population of cells comprising a plurality of RBCs provided herein, the plurality of RBCs sickle at a significantly lower oxygen tension, e.g., as measured by relative oxygen pressure, than a population of RBCs cultured from unmodified CD34+ cells, of the subject having sickle cell disease, have a significantly higher minimum elongation index upon deoxygenation than a population of RBCs cultured from unmodified CD34+ cells of the subject having sickle cell disease, and/or have a significantly higher velocity upon deoxygenation than a population of RBCs cultured from unmodified CD34+ cells of the subject having sickle cell disease.

[0040] Described 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 examples, 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 examples, a gRNA may comprise a sequence set forth in **Table 6**, **Table 12**, or **Table 13**. In certain examples, a gRNA may comprise a gRNA targeting domain. In certain examples, 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 examples, a gRNA may comprise a gRNA sequence set forth in **Table 13**. In certain examples, a gRNA may comprise a sequence selected from the group consisting of SEQ ID NOs:1022, 1023, 1041-1105. In certain examples, the RNP complex may comprise an RNP complex set forth in **Table 15**. 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 15**).

[0041] 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 examples, the modified Cpf1 protein may contain one or more modifications. In certain examples, 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 examples, 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 examples, the gRNA may be a modified or unmodified gRNA. In certain examples, the gRNA may comprise a sequence set forth in **Table 6**, **Table 12**, or **Table 13**. In certain examples, the RNP complex may comprise an RNP complex set forth in **Table 15**. 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 15**). In certain examples, an RNP complex comprising a modified Cpf1 protein may increase editing of a target nucleic acid. In certain examples, an RNP complex comprising a modified Cpf1 protein may increase editing resulting in an increase of productive indels. In various examples, 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 the target nucleic acid and subsequent sequencing analysis (e.g., Sanger sequencing, next generation sequencing).

[0042] The inventors have also discovered herein that delivery of an RNP complex including a modified gRNA complexed to an unmodified or modified Cpf1 protein may result in increased editing of a target nucleic acid. In certain examples, the modified gRNA may comprise one or more modifications including a phosphorothioate linkage modification, a phosphorodithioate (PS2) linkage modification, a 2'-O-methyl modification, one or more or a stretch of deoxyribonucleic acid (DNA) bases (also referred to herein as a "DNA extension"), one or more or a stretch of ribonucleic acid (RNA) bases (also referred to herein as a "RNA extension"), or combinations thereof. In certain examples, the DNA extension may comprise a sequence set forth in **Table 18**. For example, in certain examples, the DNA extension may comprise a sequence set forth in SEQ ID NOs:1235-1250. In certain examples, the RNA extension may comprise a sequence set forth in **Table 18**. For example, in certain examples, the RNA extension may comprise a sequence set forth in SEQ ID NOs:1231-1234, 1251-1253. In certain examples, the gRNA may comprise a sequence set forth in **Table 6**, **Table 12**, or **Table 13**. In certain examples, the RNP complex may comprise an RNP complex set forth in **Table 15**. 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 15**). In certain examples, an RNP complex comprising

a modified gRNA may increase editing of a target nucleic acid. In certain examples, an RNP complex comprising a modified gRNA may increase editing resulting in an increase of productive indels.

[0043] In certain examples, an RNP complex comprising a modified gRNA and a modified Cpf1 protein may increase editing of a target nucleic acid. In certain examples, an RNP complex comprising a modified gRNA and a modified Cpf1 protein may increase editing resulting in an increase of productive indels.

[0044] The inventors have also discovered that codelivery of an RNP complex comprising a gRNA complexed to a Cpf1 molecule (e.g., "gRNA-Cpf1-RNP") with a "booster element" may result in increased editing of a target nucleic acid. In certain examples, the RNP complex may comprise an RNP complex set forth in **Table 15**. 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 15**). As used herein, the term "booster element" refers to an element which, when co-delivered with a RNP complex comprising a gRNA complexed to an RNA-guided nuclease ("gRNA-nuclease-RNP"), increases editing of a target nucleic acid compared with editing of the target nucleic acid without the booster element. In certain examples, one or more booster elements may be codelivered with a gRNA-nuclease-RNP complex to increase editing of a target nucleic acid. In certain examples, codelivery of a booster element may increase editing resulting in an increase of productive indels. In various examples, 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 the target nucleic acid and subsequent sequencing analysis (e.g., Sanger sequencing, next generation sequencing).

[0045] In certain examples, a gRNA-nuclease-RNP may comprise a gRNA-Cpf1-RNP. In certain examples, a Cpf1 molecule of the gRNA-Cpf1-RNP complex may be a wild-type Cpf1 or modified Cpf1. In certain examples, the Cpf1 molecule of the gRNA-Cpf1-RNP 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 examples, the gRNA-Cpf1-RNP complex may comprise a gRNA comprising a targeting domain set forth in **Table 6** or **Table 12**. In certain examples, the gRNA-Cpf1-RNP complex may comprise a gRNA comprising a sequence set forth in **Table 13**. In certain examples, the gRNA may be a modified or unmodified gRNA.

[0046] In certain examples, a booster element may comprise a dead RNP comprising a dead gRNA molecule complexed with an RNA-guided nuclease molecule ("dead gRNA-nuclease-RNP"). In certain examples, the dead gRNA-nuclease-RNP may comprise a dead gRNA complexed with a wild-type (WT) Cas9 molecule ("dead gRNA-Cas9-RNP"), a dead gRNA complexed with a Cas9 nickase molecule ("dead gRNA-nickase-RNP") or a dead gRNA complexed with an enzymatically

inactive (ei) Cas9 molecule ("dead gRNA-eiCas9-RNP"). In certain examples, the dead gRNA-nuclease-RNP complex may have decreased activity or lack nuclease activity. In certain examples, the dead gRNA of the dead gRNA-nuclease-RNP complex may comprise any of the dead gRNAs set forth herein. For example, the dead gRNA may comprise a targeting domain may be the same as or may differ by no more than 3 nucleotides from a dead gRNA targeting domain set forth in **Table 8** or **Table 9**. In certain examples, the dead gRNA may include a targeting domain comprising a truncation of a gRNA targeting domain. In certain examples, the gRNA targeting domain to be truncated may be a gRNA targeting domain set forth in **Table 8** or **Table 9**. In certain examples, the dead gRNA may be a modified or unmodified dead gRNA. As shown herein, codelivery of a gRNA-Cpf1-RNP with a dead gRNA-Cas9-RNP (i.e., an RNP comprising a dead gRNA complexed to a WT Cas9) or codelivery of a gRNA-Cpf1-RNP with a dead gRNA-nickase-RNP (i.e., an RNP comprising a dead gRNA complexed to a Cas9 nickase (i.e., the Cas9 D10A nickase)) resulted in an increase in total editing above levels observed following delivery of gRNA-Cpf1-RNP alone (see, e.g., **Examples 5, 7, 8**). Dead gRNA molecules may comprise targeting domains complementary to regions proximal to or within a target region (e.g., the CCAAT box target region, 13 nt target region, proximal HBG1/2 promoter target sequence, and/or the GATA1 binding motif in BCL11Ae) in a target nucleic acid. In certain examples, "proximal to" may denote the region within 10, 25, 50, 100, or 200 nucleotides of a target region (e.g., the CCAAT box target region, 13 nt target region, proximal HBG1/2 promoter target sequence, and/or the GATA1 binding motif in BCL11Ae). In certain embodiments, one or more booster elements may be comprised of one or more dead gRNA-nuclease-RNPs, e.g., dead gRNA-Cas9-RNP, dead gRNA-nickase-RNP, dead gRNA-eiCas9-RNP, to be codelivered with a gRNA-Cpf1-RNP. In certain examples, codelivery of a dead gRNA-nuclease-RNP does not alter the indel profile of a gRNA-Cpf1-RNP.

[0047] In certain examples, a booster element may comprise an RNP complex comprising a gRNA molecule complexed with an RNA-guided nuclease nickase molecule ("gRNA-nickase-RNP"). In certain examples, the RNA-guided nuclease nickase molecule may be a Cas9 nickase molecule, e.g., Cas9 D10A nickase. In certain examples, the gRNA of the gRNA-nickase-RNP may comprise any of the gRNAs set forth herein. For example, the gRNA may comprise a gRNA targeting domain set forth in **Table 8** or **Table 9**. In certain examples, the gRNA may be a modified or unmodified gRNA. As shown herein, codelivery of gRNA-Cpf1-RNP with a gRNA-nickase-RNP complex (RNP comprising a guide RNA complexed to a Cas9D10A nickase molecule) resulted in an increase in total editing above levels observed following delivery of gRNA-Cpf1-RNP alone (see, e.g., **Examples 4, 5**). Additionally, codelivery of a gRNA-nickase-RNP complex with a gRNA-Cpf1-RNP complex altered the directionality, length, and/or position of the indel profile of gRNA-Cpf1-RNP. In certain examples, a booster enhancer may be used to provide a desired editing outcome, for example, to increase the rate of productive indels. In certain examples,

codelivery of a gRNA-nickase-RNP complex with a gRNA-Cpf1-RNP complex may alter the indel profile of the gRNA-Cpf1-RNP.

[0048] In certain examples, a booster element may comprise a single stranded oligodeoxynucleotide (ssODN) or a double stranded oligodeoxynucleotide (dsODN). In certain examples, the ssODN may be any ssODN disclosed herein. In certain examples, an ssODN may comprise a sequence set forth in **Table 7**. For example, in certain examples, an ssODN may comprise the sequence set forth in SEQ ID NO:1040.

[0049] In one example, there is described an RNP complex comprising a CRISPR from Prevotella and Franciscella 1 (Cpf1) RNA-guided nuclease or a variant thereof and a gRNA, wherein the gRNA is capable of binding to a target site in a promoter of an *HBG* gene in a cell. In certain examples, the gRNA may be modified or unmodified. In certain examples, the gRNA may comprise one or more modifications including a phosphorothioate linkage modification, a phosphorodithioate (PS2) linkage modification, a 2'-O-methyl modification, a DNA extension, an RNA extension, or combinations thereof. In certain examples, the DNA extension may comprise a sequence set forth in **Table 18**. In certain examples, the RNA extension may comprise a sequence set forth in **Table 18**. In certain examples, the gRNA may comprise a sequence set forth in **Table 6**, **Table 12**, or **Table 13**. In certain examples, the RNP complex may comprise an RNP complex set forth in **Table 15**. For example, the RNP complex may include a gRNA comprising the sequence set forth in SEQ ID NO:1051 and a Cpf1 variant protein encoded by the sequence set forth in SEQ ID NO:1097 (RNP32, **Table 15**). In certain examples, the Cpf1 variant protein may contain one or more modifications. In certain examples, 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 examples, a Cpf1 variant protein 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).

[0050] In one example, there is described a method of altering a promoter of an *HBG* gene in a cell comprising contacting the cell with an RNP complex disclosed herein. In certain examples, the alteration may comprise an indel within one or more regions set forth in **Table 11**. In certain examples, the alteration may comprise an indel within a CCAAT box target region of the promoter of an *HBG* gene. For example, in certain examples, the alteration may comprise an indel within Chr 11 (NC_000011.10): 5,249,955 – 5,249,987 (**Table 11**, Region 6), Chr 11 (NC_000011.10): 5,254,879 – 5,254,909 (**Table 11**, Region 16), or a combination thereof. In certain examples, the RNP complex may comprise a gRNA and a Cpf1 protein. In certain examples, the gRNA may comprise an RNA targeting domain set forth in **Table 13**. In certain examples, the

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 examples, the gRNA may comprise a gRNA sequence set forth in **Table 13**. In certain examples, the gRNA may comprise a sequence selected from the group consisting of SEQ ID NOs:1022, 1023, 1041-1105. In certain examples, a gRNA may be configured to provide an editing event at Chr11:5249973, Chr11:5249977 (HBG1); Chr11:5250042, Chr11:5250046 (HBG1); Chr11:5250055, Chr11:5250059 (HBG1); Chr11:5250179, Chr11:5250183 (HBG1); Chr11:5254897, Chr11:5254901 (HBG2); Chr11:5254897, Chr11:5254901 (HBG2); Chr11:5254966, 5254970 (HBG2); Chr11:5254979, 5254983 (HBG2) (**Table 16**, **Table 17**). In certain examples, the cell may be further contacted with a booster element. In certain examples, a booster element may comprise a single stranded oligodeoxynucleotide (ssODN) or a double stranded oligodeoxynucleotide (dsODN). In certain examples, the ssODN may be any ssODN disclosed herein. In certain examples, an ssODN may comprise a sequence set forth in **Table 7**. For example, in certain examples, an ssODN may comprise the sequence set forth in SEQ ID NO:1040.

[0051] In one example, there is described an isolated cell comprising an alteration in a promoter of *HBG* gene generated by the delivery of an RNP complex to the cell. In certain examples, the RNP complex may comprise a gRNA and a Cpf1 protein. In certain examples, the gRNA may be modified or unmodified. In certain examples, the gRNA may comprise one or more modifications including a phosphorothioate linkage modification, a phosphorodithioate (PS2) linkage modification, a 2'-O-methyl modification, a DNA extension, an RNA extension, or combinations thereof. In certain examples, the DNA extension may comprise a sequence set forth in **Table 18**. In certain examples, the RNA extension may comprise a sequence set forth in **Table 18**. In certain examples, the gRNA may comprise a sequence set forth in **Table 6**, **Table 12**, or **Table 13**. In certain examples, the RNP complex may comprise an RNP complex set forth in **Table 15**. For example, the RNP complex may include a gRNA comprising the sequence set forth in SEQ ID NO:1051 and a Cpf1 variant protein encoded by the sequence set forth in SEQ ID NO:1097 (RNP32, **Table 15**). In certain examples, the Cpf1 variant protein may contain one or more modifications. In certain examples, 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 examples, a Cpf1 variant protein 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 examples, a booster element may be co-delivered with the RNP complex. In certain examples, a booster element may comprise a single stranded oligodeoxynucleotide (ssODN) or a double stranded oligodeoxynucleotide (dsODN). In certain examples, the ssODN

may be any ssODN disclosed herein. In certain examples, an ssODN may comprise a sequence set forth in **Table 7**. For example, in certain examples, an ssODN may comprise the sequence set forth in SEQ ID NO:1040.

[0052] In one example, there is disclosed 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 gRNA and a Cpf1 RNA-guided nuclease or a variant thereof to affect an alteration in a promoter of an *HBG* gene, thereby to increase expression of HbF. In certain examples, the gRNA may be modified or unmodified. In certain examples, the gRNA may comprise one or more modifications including a phosphorothioate linkage modification, a phosphorodithioate (PS2) linkage modification, a 2'-O-methyl modification, a DNA extension, an RNA extension, or combinations thereof. In certain examples, the DNA extension may comprise a sequence set forth in **Table 18**. In certain examples, the RNA extension may comprise a sequence set forth in **Table 18**. In certain examples, the gRNA may comprise a sequence set forth in **Table 6**, **Table 12**, or **Table 13**. In certain examples, the RNP complex may comprise an RNP complex set forth in **Table 15**. For example, the RNP complex may include a gRNA comprising the sequence set forth in SEQ ID NO:1051 and a Cpf1 variant protein encoded by the sequence set forth in SEQ ID NO:1097 (RNP32, **Table 15**). In certain examples, the Cpf1 variant protein may contain one or more modifications. In certain examples, 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 examples, a Cpf1 variant protein 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 examples, a booster element may be co-delivered with the RNP complex. In certain examples, a booster element may comprise a single stranded oligodeoxynucleotide (ssODN) or a double stranded oligodeoxynucleotide (dsODN). In certain examples, the ssODN may be any ssODN disclosed herein. In certain examples, an ssODN may comprise a sequence set forth in **Table 7**. For example, in certain examples, an ssODN may comprise the sequence set forth in SEQ ID NO:1040.

[0053] In one example, there is disclosed a population of CD34+ or hematopoietic stem cells, wherein one or more cells in the population comprises an alteration in a promoter of an *HBG* gene, which alteration is generated by delivering an RNP complex comprising a gRNA and a Cpf1 RNA-guided nuclease or a variant thereof to the population of CD34+ or hematopoietic stem cells. In certain examples, the gRNA may be modified or unmodified. In certain examples, the gRNA may comprise one or more modifications including a phosphorothioate linkage modification, a phosphorodithioate (PS2) linkage modification, a 2'-O-methyl modification, a DNA extension, an

RNA extension, or combinations thereof. In certain examples, the DNA extension may comprise a sequence set forth in **Table 18**. In certain examples, the RNA extension may comprise a sequence set forth in **Table 18**. In certain examples, the gRNA may comprise a sequence set forth in **Table 6**, **Table 12**, or **Table 13**. In certain examples, the RNP complex may comprise an RNP complex set forth in **Table 15**. For example, the RNP complex may include a gRNA comprising the sequence set forth in SEQ ID NO:1051 and a Cpf1 variant protein encoded by the sequence set forth in SEQ ID NO:1097 (RNP32, **Table 15**). In certain examples, the Cpf1 variant protein may contain one or more modifications. In certain examples, 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 examples, a Cpf1 variant protein 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 examples, a booster element may be co-delivered with the RNP complex. In certain examples, a booster element may comprise a single stranded oligodeoxynucleotide (ssODN) or a double stranded oligodeoxynucleotide (dsODN). In certain examples, the ssODN may be any ssODN disclosed herein. In certain examples, an ssODN may comprise a sequence set forth in **Table 7**. For example, in certain examples, an ssODN may comprise the sequence set forth in SEQ ID NO:1040.

[0054] In one example, there is described a method of alleviating one or more symptoms of sickle cell disease in a subject in need thereof, the method comprising: a) isolating a population of CD34+ or hematopoietic stem cells from the subject; b) modifying the population of isolated cells ex vivo by delivering an RNP complex comprising a gRNA and a Cpf1 RNA-guided nuclease or a variant thereof to the population of isolated cells, thereby to affect an alteration in a promoter of an *HBG* gene in one or more cells in the population; and c) administering the modified population of cells to the subject, thereby to alleviate one or more symptoms of sickle cell disease in the subject. In certain examples, the method may further comprise detecting progeny/daughter cells of the administered modified cells in the subject, e.g., in the form of BM-engrafted CD34+ hematopoietic stem cells or blood cells derived from those (e.g., myeloid progenitor or differentiated myeloid cells (e.g., erythrocyte, mast cells, myoblast); or lymphoid progenitors or differentiated lymphoid cells (e.g., T- or B- lymphocyte, or NK cell), e.g., at least [1, 2, 3, 4, 5, 6, 7, 8, 12, 16, or 20] weeks, or at least [1, 2, 3, 4, 5, or 6] months, or at least [1, 2, 3, 4, or 5] years after administration. In certain examples, the method may result in a reconstitution of all hematopoietic cell lineages, e.g., without any differentiation bias, e.g., without an erythroid lineage differentiation bias. In certain examples, the method may comprise administering a plurality of edited cells, and the method may result in long-term engraftment [e.g., at least [1, 2, 3, 4, 5, 6, 7, 8, 12, 16, or 20] weeks, or at least [1,

2, 3, 4, 5, or 6] months, or at least [1, 2, 3, 4, or 5] years after administration] of a plurality of [at least 5, 10, 15, 20, 25, … 100] different HSC clones in the BM. In certain examples, the method may further comprise detecting the level of total hemoglobin expression in the subject, at least [1, 2, 3, 4, 5, 6, 7, 8, 12, 16, or 20] weeks, or at least [1, 2, 3, 4, 5, or 6] months, or at least [1, 2, 3, 4, or 5] years after administration. In certain examples, the method may result in long-term expression [e.g., at least [1, 2, 3, 4, 5, 6, 7, 8, 12, 16, or 20] weeks, or at least [1, 2, 3, 4, 5, or 6] months, or at least [1, 2, 3, 4, or 5] years after administration] of [at least 50%, at least 60%... at least 99%] of total hemoglobin as compared to a healthy subject (e.g., as total Hb (e.g., HbA and HbF (if any) combined)). In certain examples, the alteration may comprise an indel within a CCAAT box target region of the promoter of the HBG gene. In certain examples, the RNP complex may be delivered using electroporation. In certain examples, at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80% or at least about 90% of the cells in the population of cells comprise a productive indel.

[0055] In one example, there is described a gRNA comprising a 5' end and a 3' end, and comprising a DNA extension at the 5' end and a 2'-O-methyl-3'-phosphorothioate modification at the 3' end, wherein the gRNA includes an RNA segment capable of hybridizing to a target site and an RNA segment capable of associating with a Cpf1 RNA-guided nuclease. In certain examples, the DNA extension may comprise a sequence set forth in SEQ ID NOs:1235-1250. In certain examples, the gRNA may be modified or unmodified. In certain examples, the gRNA may comprise one or more modifications including a phosphorothioate linkage modification, a phosphorodithioate (PS2) linkage modification, a 2'-O-methyl modification, a DNA extension, an RNA extension, or combinations thereof. In certain examples, the DNA extension may comprise a sequence set forth in **Table 18**. In certain examples, the RNA extension may comprise a sequence set forth in **Table 18**. In certain examples, the gRNA may comprise a sequence set forth in **Table 6**, **Table 12**, or **Table 13**.

[0056] In one example, there is described an RNP complex comprising a Cpf1 RNA-guided nuclease as disclosed herein and a gRNA as disclosed herein.

[0057] Also provided herein are genome editing systems, guide RNAs, and CRISPR-mediated methods for altering one or more γ–globin genes (e.g., *HBG1*, *HBG2*, or *HBG1* and *HBG2*) and increasing expression of fetal hemoglobin (HbF). In certain examples, one or more gRNAs comprising a sequence set forth in **Table 12** or **Table 13** may be used to introduce alterations in the promoter region of the *HBG* gene. In certain examples, genome editing systems, guide RNAs, and CRISPR-mediated methods may alter a 13 nucleotide (nt) target region that is 5' of the transcription site of the *HBG1*, *HBG2*, or *HBG1* and *HBG2* gene ("13 nt target region"). In certain examples, genome editing systems, guide RNAs, and CRISPR-mediated methods may alter a

CCAAT box target region that is 5' of the transcription site of the *HBG1*, *HBG2*, or *HBG1* and *HBG2* gene ("CCAAT box target region"). In certain examples, the CCAAT box target region may be the region that is at or near the distal CCAAT box and includes the nucleotides of the distal CCAAT box and 25 nucleotides upstream (5') and 25 nucleotides downstream (3') of the distal CCAAT box (i.e., HBG1/2 c.-86 to -140). In certain examples, the CCAAT box target region may be the region that is at or near the distal CCAAT box and includes the nucleotides of the distal CCAAT box and 5 nucleotides upstream (5') and 5 nucleotides downstream (3') of the distal CCAAT box (i.e., *HBG1/2* c.-106 to -120). In certain examples, the CCAAT box target region may comprise a 18 nt target region, a 13 nt target region, a 11 nt target region, a 4 nt target region, a 1 nt target region, a -117G>A target region, or a combination thereof as disclosed herein. In certain examples, the alteration may be a 18 nt deletion, 13 nt deletion, 11 nt deletion, 4 nt deletion, 1 nt deletion, a substitution from G to A at c.-117 of the *HBG1*, *HBG2*, or *HBG1* and *HBG2* gene, or a combination thereof. In certain examples, the alteration may be a non-naturally occurring alteration or a naturally occurring alteration.

[0058] Also provided herein in certain examples are the use of optional genome editing system components such as template nucleic acids (oligonucleotide donor templates). In certain examples, template nucleic acids for use in targeting the CCAAT target region may include, without limitation, template nucleic acids encoding alterations of the CCAAT box target region. In certain examples, the CCAAT box target region may comprise a 18 nt target region, a 11 nt target region, a 4 nt target region, a 1 nt target region, or a combination thereof. In certain examples, the template nucleic acid may be a single stranded oligodeoxynucleotide (ssODN) or a double stranded oligodeoxynucleotide (dsODN). In certain examples, 5' and 3' homology arms, and exemplary full-length donor templates encoding alterations at the CCAAT box target region are also presented below (e.g., SEQ ID NOS: 974-995, 1040). In certain examples, the template nucleic acid may be a positive strand or a negative strand. In certain examples, the ssODN may comprise a 5' homology arm, a replacement sequence, and a 3' homology arm. In certain examples, the 5' homology arm may be about 25 to about 200 nucleotides or more in length, e.g., at least about 25, 50, 75, 100, 125, 150, 175, or 200 nucleotides in length; the replacement sequence may comprise 0 nucleotides in length; and the 3' homology arm may be about 25 to about 200 nucleotides or more in length, e.g., at least about 25, 50, 75, 100, 125, 150, 175, or 200 nucleotides in length. In certain examples, the ssODN may comprise one or more phosphorothioates.

[0059] In certain examples, the genome editing systems, guide RNAs, and CRISPR-mediated methods for altering one or more γ–globin genes (e.g., *HBG1*, *HBG2*, or *HBG1* and *HBG2*), may include an RNA-guided nuclease. In certain examples, the RNA-guided nuclease may a Cpf1 or modified Cpf1 as disclosed herein.

[0060] In one example, there are described compositions including a plurality of cells generated by the methods disclosed above, in which at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the cells include an alteration of a sequence of a 13 nt target region of the human *HBG1* or *HBG2* gene or a plurality of cells generated by the methods disclosed above, wherein at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the cells include an alteration of a sequence of a 13 nt target region of the human *HBG1* or *HBG2* gene. In certain examples, at least a portion of the plurality of cells may be within an erythroid lineage. In certain examples, the plurality of cells may be characterized by an increased level of fetal hemoglobin expression relative to an unmodified plurality of cells. In certain examples, the level of fetal hemoglobin may be increased by at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90%. In certain examples, the compositions may further include a pharmaceutically acceptable carrier.

[0061] The disclosure herein also relates to methods of altering a cells, including contacting a cell with any of the genome editing systems disclosed herein. In certain examples, the step of contacting the cell may comprise contacting the cell with a solution comprising first and second ribonucleoprotein complexes. In certain examples, the step of contacting the cell with the solution further comprises electroporating the cells, thereby introducing the first and second ribonucleoprotein complexes into the cell.

[0062] A genome editing system or method including any of all of the features described above may include a target nucleic acid comprising a human *HBG1*, *HBG2* gene, or a combination thereof. In certain examples, the target region may be a CCAAT box target region of the human *HBG1*, *HBG2* gene, or a combination thereof. In certain examples, the first targeting domain sequence may be complementary to a first sequence on a side of a CCAAT box target region of the human *HBG1*, *HBG2* gene, or a combination thereof, in which the first sequence optionally overlaps the CCAAT box target region of the human *HBG1*, *HBG2* gene, or a combination thereof. In certain examples, the second targeting domain sequence may be complementary to a second sequence on a side of a CCAAT box target region of the human *HBG1*, *HBG2* gene, or a combination thereof, in which the second sequence optionally overlaps the CCAAT box target region of the human *HBG1*, *HBG2* gene, or a combination thereof. In certain examples, the first targeting domain may comprise a truncation of a gRNA targeting domain. In certain examples, the gRNA targeting domain may include the gRNAs set forth in **Table 8** or **Table 9**, and the gRNA targeting domain has been truncated from a 5' end of the gRNA targeting domain. In certain examples, the first targeting domain may be the same as or differs by no more than 3 nucleotides from a dgRNA targeting domain set forth in **Table 8** or **Table 9**. In certain examples, the second targeting domain differs by no more than 3 nucleotides from a gRNA targeting domain set forth in **Table 8** or **Table 9**. In certain examples, the indel may alter the CCAAT box target region indel. In certain examples, the indel may be a productive indel resulting in an increased level of fetal hemoglobin

expression. In certain examples, the gRNA, dgRNA, or both may be *in vitro* synthesized or chemically synthesized.

[0063] In certain examples, a cell may include at least one modified allele of the *HBG* locus generated by any of the methods for altering a cell disclosed herein, in which the modified allele of the *HBG* locus comprises an alteration of the human *HBG1* gene, *HBG2*, gene, or a combination thereof.

[0064] In certain examples, an isolated population of cells may be modified by any of the methods for altering a cells disclosed herein, wherein the population of cells may include a distribution of indels that may be different from an isolated population of cells or their progenies of the same cell type that have not been modified by the method.

[0065] In certain examples, a plurality of cells may be generated by any of the methods for altering a cells disclosed herein, in which at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the cells may include an alteration of a sequence in the CCAAT box target region of the human *HBG1* gene, *HBG2* gene or a combination thereof.

[0066] In certain examples, the cells disclosed herein may be used for a medicament. In certain examples, the cells may be for use in the treatment of β-hemoglobinopathy. In certain examples, β-hemoglobinopathy may be selected from the group consisting of sickle cell disease and beta-thalassemia.

[0067] In one example, there are described compositions including a plurality of cells generated by a method including a dgRNA disclosed above, in which at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the cells include an alteration of a sequence of a CCAAT box target region of the human *HBG1* or *HBG2* gene or a plurality of cells generated by the method disclosed above, wherein at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the cells include an alteration of a sequence of a CCAAT box target region of the human *HBG1* or *HBG2*. In certain examples, at least a portion of the plurality of cells may be within an erythroid lineage. In certain examples, the plurality of cells may be characterized by an increased level of fetal hemoglobin expression relative to an unmodified plurality of cells. In certain examples, the level of fetal hemoglobin may be increased by at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90%. In certain examples, the compositions may further include a pharmaceutically acceptable carrier.

[0068] In one example, there is described a population of cells modified by a genome editing system including a dgRNA described above, wherein the population of cells comprise a higher percentage of a productive indel relative to a population of cells not modified by the genome editing system. The disclosure also relates to a population of cells modified by the genome editing system including a dgRNA described above, wherein a higher percentage of the population of cells are capable of differentiating into a population of cells of an erythroid lineage that express HbF relative to a population of cells not modified by the genome editing system. In certain examples, the higher

percentage may be at least about 15%, at least about 20%, at least about 25%, at least about 30%, or at least about 40% higher. In certain examples, the cells may be hematopoietic stem cells. In certain examples, the cells may be capable of differentiating into an erythroblast, erythrocyte, or a precursor of an erythrocyte or erythroblast. In certain examples, the indel may be created by a repair mechanism other than microhomology-mediated end joining (MMEJ) repair.

[0069] The disclosure also describes the use of any of the cells disclosed herein in the manufacture of a medicament for treating β-hemoglobinopathy in a subject.

[0070] In one example, there is describeda method of treating a β-hemoglobinopathy in a subject in need thereof, comprising administering to the subject the cells disclosed herein. In certain examples, a method of treating a β-hemoglobinopathy in a subject in need thereof, may include administering a population of modified hematopoietic cells to the subject, wherein one or more cells have been altered according to the methods of altering a cell disclosed herein. In certain examples, the method may further comprise detecting progeny/daughter cells of the administered modified cells in the subject, e.g., in the form of BM-engrafted CD34+ hematopoietic stem cells or blood cells derived from those (e.g., myeloid progenitor or differentiated myeloid cells (e.g., erythrocyte, mast cells, myoblast); or lymphoid progenitors or differentiated lymphoid cells (e.g., T- or B- lymphocyte, or NK cell), e.g., at least [1, 2, 3, 4, 5, 6, 7, 8, 12, 16, or 20] weeks, or at least [1, 2, 3, 4, 5, or 6] months, or at least [1, 2, 3, 4, or 5] years after administration. In certain examples, the method may result in a reconstitution of all hematopoietic cell lineages, e.g., without any differentiation bias, e.g., without an erythroid lineage differentiation bias. In certain examples, the method may comprise administering a plurality of edited cells, and the method may result in long-term engraftment [e.g., at least [1, 2, 3, 4, 5, 6, 7, 8, 12, 16, or 20] weeks, or at least [1, 2, 3, 4, 5, or 6] months, or at least [1, 2, 3, 4, or 5] years after administration] of a plurality of [at least 5, 10, 15, 20, 25, … 100] different HSC clones in the BM. In certain examples, the method may further comprise detecting the level of total hemoglobin expression in the subject, at least [1, 2, 3, 4, 5, 6, 7, 8, 12, 16, or 20] weeks, or at least [1, 2, 3, 4, 5, or 6] months, or at least [1, 2, 3, 4, or 5] years after administration. In certain examples, the method may result in long-term expression [e.g., at least [1, 2, 3, 4, 5, 6, 7, 8, 12, 16, or 20] weeks, or at least [1, 2, 3, 4, 5, or 6] months, or at least [1, 2, 3, 4, or 5] years after administration] of [at least 50%, at least 60%... at least 99%] of total hemoglobin as compared to a healthy subject (e.g., as total Hb (e.g., HbA and HbF (if any) combined)). In certain examples, the alteration may comprise an indel within a CCAAT box target region of the promoter of the HBG gene.

[0071] In one example, there is described a genome editing system, comprising: an RNA-guided nuclease; and a first guide RNA, in which the first guide RNA may comprise a first targeting domain that is complementary to a first sequence on a side of a CCAAT box target region of a human *HBG1, HBG2* gene, or a combination thereof, in which the first sequence optionally overlaps the CCAAT

box target region of the human *HBG1, HBG2* gene, or a combination thereof. In certain examples, the genome editing system may further comprise a template nucleic acid encoding an alteration of the CCAAT box target region of a human *HBG1, HBG2* gene, or a combination thereof. In certain examples, the template nucleic acid may be a single stranded oligodeoxynucleotide (ssODN) or a double stranded oligodeoxynucleotide (dsODN). In certain examples, the ssODN may comprise a 5' homology arm, a replacement sequence, and a 3' homology arm. In certain examples, the homology arms may be symmetrical in length. In certain examples, the homology arms may be asymmetrical in length. In certain examples, the ssODN may comprise one or more phosphorothioate modifications. In certain examples, the one or more phosphorothioate modifications may be at the 5' end, the 3' end or a combination thereof. In certain examples, the ssODN may be a positive or negative strand. In certain examples, the alteration may be a non-naturally occurring alteration. In certain examples, the alteration may comprise a deletion of the CCAAT box target region. In certain examples, the deletion may comprise a 18 nt deletion, a 11 nt deletion, a 4 nt deletion, a 1 nt deletion, or a combination thereof. In certain examples, the CCAAT box target region may comprise a 18 nt target region, a 11 nt target region, a 4 nt target region, a 1 nt target region, or a combination thereof. In certain examples, the 5' homology arm may be about 25 to about 200 or more nucleotides in length, e.g., at about least 25, 50, 75, 100, 125, 150, 175, or 200 nucleotides in length; the replacement sequence may comprise 0 nucleotides in length; and the 3' homology arm may be about 25 to about 200 or more nucleotides in length, e.g., at least about 25, 50, 75, 100, 125, 150, 175, or 200 nucleotides in length. In certain examples, the 5' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 5' of the 18 nt target region and the 3' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 3' of the 18 nt target region. In certain examples, the ssODN may comprise, may consist essentially of, or may consist of SEQ ID NO:974 or SEQ ID NO:975. In certain examples, the 5' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 5' of the 11 nt target region and the 3' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 3' of the 11 nt target region. In certain examples, the ssODN may comprise, may consist essentially of, or may consist of SEQ ID NO:976 or SEQ ID NO:978. In certain examples, the 5' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 5' of the 4 nt target region and the 3' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 3' of the 4 nt target region. In certain examples, the ssODN may comprise, may consist essentially of, or may consist of a sequence selected from the group consisting of SEQ ID NO:984, SEQ ID NO:985, SEQ ID NO:986, SEQ ID NO:987, SEQ ID NO:988, SEQ ID NO:989, SEQ ID NO:990, SEQ ID NO:991, SEQ ID NO:992, SEQ ID NO:993, SEQ ID NO:994, and SEQ ID NO:995. In certain examples,

the 5' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 5' of the 1 nt target region and the 3' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 3' of the 1 nt target region. In certain examples, the homology arms may be symmetrical in length. In certain examples, the ssODN may comprise, may consist essentially of, or may consist of SEQ ID NO:982 or SEQ ID NO:983. In certain examples, the alteration may be a naturally occurring alteration. In certain examples, the alteration may comprise a deletion or mutation of the CCAAT box target region. In certain examples, the CCAAT box target region may comprise a 13 nt target region, -117G>A target region, or a combination thereof. In certain examples, the alteration may comprise a 13 nt deletion at the 13 nt target region or a substitution from G to A at the -117G>A target region, or a combination thereof. In certain examples, the 5' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 5' of the 13 nt target region and the 3' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 3' of the 13 nt target region. In certain examples, the ssODN may comprise, may consist essentially of, or may consist of SEQ ID NO:977 or SEQ ID NO:979. In certain examples, the 5' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 5' of the 13 nt target region and the 3' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 3' of the 13 nt target region. In certain examples, the ssODN may comprise, may consist essentially of, or may consist of SEQ ID NO:980 or SEQ ID NO:981. In certain examples, the RNA-guided nuclease may be an *S. pyogenes* Cas9. In certain examples, the RNA-guided nuclease may be a Cpf1 variant as disclosed herein. In certain examples, the first targeting domain may differ by no more than 3 nucleotides from a targeting domain listed in **Table 12, Table 13** or a gRNA in **Table 13**. In certain examples, the genome editing system may further comprise a second guide RNA, wherein the second guide RNA may comprise a second targeting domain that may be complementary to a second sequence on a side of a CCAAT box target region of a human *HBG1, HBG2* gene, or a combination thereof, wherein the second sequence optionally overlaps the CCAAT box target region of the human *HBG1, HBG2* gene, or a combination thereof. In certain examples, the RNA-guided nuclease may be a nickase, and optionally lacks RuvC activity. In certain examples, the genome editing system may comprise first and second RNA-guided nucleases. In certain examples, the first and second RNA-guided nucleases may be complexed with the first and second guide RNAs, respectively, forming first and second ribonucleoprotein complexes. In certain examples, the genome editing system may further comprise a third guide RNA; and optionally a fourth guide RNA, wherein the third and fourth guide RNAs may comprise third and fourth targeting domains complimentary to third and fourth sequences on opposite sides of positions of a GATA1 binding motif in *BCL11A* erythroid enhancer (*BCL11Ae*) of a human *BCL11A* gene, wherein one or both of the third and fourth sequences

optionally overlaps the GATA1 binding motif in *BCL11Ae* of the human *BCL11A* gene. In certain examples, the genome editing system may further comprise a nucleic acid template encoding a deletion of the GATA1 binding motif in *BCL11Ae*. In certain examples, the RNA-guided nuclease may be an *S. pyogenes* Cas9. In certain examples, the RNA-guided nuclease may be a nickase, and optionally lacks RuvC activity. In certain examples, the third targeting domain may be complimentary to a sequence within 1000 nucleotides upstream of the GATA1 binding motif in *BCL11Ae*. In certain examples, the third targeting domain may be complimentary to a sequence within 100 nucleotides upstream of the GATA1 binding motif in *BCL11Ae*. In certain examples, one of the third and fourth targeting domains may be complimentary to a sequence within 100 nucleotides downstream of the GATA1 binding motif in *BCL11Ae*. In certain examples, the fourth targeting domain may be complimentary to a sequence within 50 nucleotides downstream of the GATA1 binding motif in *BCL11Ae*. In certain examples, genome editing system may comprise first and second RNA-guided nucleases. In certain examples, the first and second RNA-guided nucleases may be complexed with the third and fourth guide RNAs, respectively, forming third and fourth ribonucleoprotein complexes.

[0072] In one example, there is described a method of altering a cell comprising contacting a cell with a genome editing system. In certain examples, the step of contacting the cell with the genome editing system may comprise contacting the cell with a solution comprising first and second ribonucleoprotein complexes. In certain examples, the step of contacting the cell with the solution may further comprise electroporating the cells, thereby introducing the first and second ribonucleoprotein complexes into the cell. In certain examples, the method of altering a cell may further comprise contacting the cell with a genome editing system, wherein the step of contacting the cell with the genome editing system may comprise contacting the cell with a solution comprising first, second, third, and optionally, fourth ribonucleoprotein complexes. In certain examples, the step of contacting the cell with the solution may further comprise electroporating the cells, thereby introducing the first, second, third, and optionally, fourth ribonucleoprotein complexes into the cell. In certain examples, the cell may be capable of differentiating into an erythroblast, erythrocyte, or a precursor of an erythrocyte or erythroblast. In certain examples, the cell may be a $CD34^+$ cell.

[0073] In one example, there is described a CRISPR-mediated method of altering a cell, comprising: introducing a first DNA single strand break (SSB) or double strand break (DSB) within a genome of the cell between positions c.-106 to -120 of a human *HBG1* or *HBG2* gene; and optionally introducing a second SSB or DSB within the genome of the cell between positions c.-106 to -120 of the human *HBG1* or *HBG2* gene, wherein the first and second SSBs or DSBs may be repaired by the cell in a manner that alters a CCAAT box target region of the human *HBG1* or *HBG2* gene. In certain examples, the first and second SSBs or DSBs may be repaired by the cell in a manner that results in the alteration of a CCAAT box target region of the human *HBG1* or *HBG2* gene. In certain

examples, the CRISPR-mediated method may further comprise a template nucleic acid encoding the alteration of the CCAAT box target region of a human *HBG1, HBG2* gene, or a combination thereof. In certain examples, the template nucleic acid may be a single stranded oligodeoxynucleotide (ssODN). In certain examples, the ssODN may comprise a 5' homology arm, a replacement sequence, and a 3' homology arm. In certain examples, the ssODNs may be a positive or negative strand. In certain examples, the alteration may be a non-naturally occurring alteration. In certain examples, the first and second SSBs or DSBs may be repaired by the cell in a manner that results in the formation of at least one of an indel, a deletion, or an insertion in the CCAAT box target region of the human *HBG1* or *HBG2* gene. In certain examples, the CCAAT box target region may comprise a 18 nt target region, a 11 nt target region, a 4 nt target region, a 1 nt target region, or a combination thereof. In certain examples, the 5' homology arm may be about 25 to about 200 nucleotides or more in length, e.g., at least about 25, 50, 75, 100, 125, 150, 175, or 200 nucleotides in length; the replacement sequence may comprise 0 nucleotides in length; and the 3' homology arm may be about 25 to about 200 nucleotides or more in length, e.g., at least about 25, 50, 75, 100, 125, 150, 175, or 200 nucleotides in length. In certain examples, the 5' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 5' of the 18 nt target region, the 11 nt target region, the 4 nt target region, or the 1 nt target region and the 3' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 3' of 18 nt target region, the 11 nt target region, the 4 nt target region, or the 1 nt target region. In certain examples, the ssODN may comprise, may consist essentially of, or may consist of a sequence selected from the group consisting of SEQ ID NO:974, SEQ ID NO:975, SEQ ID NO:976, SEQ ID NO:978, SEQ ID NO:984, SEQ ID NO:985, SEQ ID NO:986, SEQ ID NO:987, SEQ ID NO:988, SEQ ID NO:989, SEQ ID NO:990, SEQ ID NO:991, SEQ ID NO:992, SEQ ID NO:993, SEQ ID NO:994, SEQ ID NO:995, SEQ ID NO:982 and SEQ ID NO:983. In certain examples, the alteration may be a non-naturally occurring alteration. In certain examples, the first and second SSBs or DSBs may be repaired by the cell in a manner that results in the formation of at least one of an indel, a deletion, or an insertion in the CCAAT box target region of the human *HBG1* or *HBG2* gene. In certain examples, the CCAAT box target region may comprise a 13 nt target region, -117G>A target region, or a combination thereof. In certain examples, the alteration may comprise a 13 nt deletion at the 13 nt target region or a substitution from G to A at the -117G>A target region, or a combination thereof. In certain examples, the 5' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 5' of the 13 nt target region or the -117G>A target region and the 3' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 3' of the 13 nt target region or the -117G>A target region. In certain examples, the ssODN may comprise, may consist essentially of,

or may consist of a sequence selected from the group consisting of SEQ ID NO:977 or SEQ ID NO:979. SEQ ID NO:980 or SEQ ID NO:981.

[0074] In one example, there is described a composition that may comprise a plurality of cells generated by a method of altering a cell disclosed herein, wherein at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the cells may comprise an alteration of a sequence of a CCAAT box target region of the human *HBG1* gene, *HBG2* gene, or a combination thereof. In certain examples, the alteration may comprise a 18 nt deletion, a 11 nt deletion, a 4 nt deletion, a 1 nt deletion, a 13 nt deletion, a substitution from G to A at the -117, of the human *HBG1* gene, *HBG2* gene, or a combination thereof. In certain examples, at least a portion of the plurality of cells may be within an erythroid lineage. In certain examples, the plurality of cells may be characterized by an increased level of fetal hemoglobin expression relative to an unmodified plurality of cells. In certain examples, the level of fetal hemoglobin may be increased by at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90%. In certain examples, the composition may further comprise a pharmaceutically acceptable carrier.

[0075] In one example, there is described a cell comprising a synthetic genotype generated by a method of altering a cell disclosed herein, wherein the cell may comprise a 18 nt deletion, a 11 nt deletion, a 4 nt deletion, a 1 nt deletion, a 13 nt deletion, a substitution from G to A at the -117, of the human *HBG1* gene, *HBG2* gene, or a combination thereof.

[0076] In one example, there is described a cell comprising at least one allele of the *HBG* locus generated by a method of altering a cell disclosed herein, wherein the cell may encode a 18 nt deletion, a 11 nt deletion, a 4 nt deletion, a 1 nt deletion, a 13 nt deletion, a substitution from G to A at the -117, of the human *HBG1* gene, *HBG2* gene, or a combination thereof.

[0077] In one example, there is described an AAV vector that may comprise a template nucleic acid encoding a non-naturally occurring alteration of a CCAAT box target region of a human *HBG1, HBG2* gene, or a combination thereof. In certain examples, the template nucleic acid may be a single stranded oligodeoxynucleotide (ssODN). In certain examples, the CCAAT box target region may comprise a 18 nt target region, a 11 nt target region, a 4 nt target region, a 1 nt target region, or a combination thereof. In certain examples, the ssODN may comprise a 5’ homology arm, a replacement sequence, and a 3’ homology arm. In certain examples, the 5’ homology arm may be about 25 to about 200 or more nucleotides in length, e.g., at least about 25, 50, 75, 100, 125, 150, 175, or 200 nucleotides in length; the replacement sequence may comprise 0 nucleotides in length; and the 3’ homology arm may be about 25 to about 200 or more nucleotides in length, e.g., at least about 25, 50, 75, 100, 125, 150, 175, or 200 nucleotides in length. In certain examples, the 5’ homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 5’ of the 18 nt target region, the 11 nt target region, the 4 nt target region, or the 1 nt target region and the 3’ homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90,

or 80 to 90 bp, homology 3' of 18 nt target region, the 11 nt target region, the 4 nt target region, or the 1 nt target region. In certain examples, the ssODN may comprise, may consist essentially of, or may consist of a sequence selected from the group consisting of SEQ ID NO:974-976, SEQ ID NO:978, SEQ ID NO:982-995.

[0078] In one example, there is described a nucleotide sequence comprising a template nucleic acid encoding a non-naturally occurring alteration of a CCAAT box target region of a human *HBG1, HBG2* gene, or a combination thereof. In certain examples, the template nucleic acid may be a single stranded oligodeoxynucleotide (ssODN) or a double stranded oligodeoxynucleotide (dsODN) comprising the alteration. In certain examples, the CCAAT box target region may comprise a 18 nt target region, a 11 nt target region, a 4 nt target region, a 1 nt target region, or a combination thereof. In certain examples, the ssODN may comprise a 5' homology arm, a replacement sequence, and a 3' homology arm. In certain examples, the 5' homology arm may be about 25 to about 200 or more nucleotides in length, e.g., at least about 25, 50, 75, 100, 125, 150, 175, or 200 nucleotides in length; the replacement sequence may comprise 0 nucleotides in length; and the 3' homology arm may be about 25 to about 200 or more nucleotides in length, e.g., at least about 25, 50, 75, 100, 125, 150, 175, or 200 nucleotides in length. In certain examples, the 5' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 5' of the 18 nt target region, the 11 nt target region, the 4 nt target region, or the 1 nt target region and the 3' homology arm may comprise about 50 to 100 bp, e.g., 55 to 95, 60 to 90, 70 to 90, or 80 to 90 bp, homology 3' of 18 nt target region, the 11 nt target region, the 4 nt target region, or the 1 nt target region. In certain examples, the ssODN may comprise, may consist essentially of, or may consist of a sequence selected from the group consisting of SEQ ID NO:974-976, SEQ ID NO:978, SEQ ID NO:982-995.

[0079] In one example, there is described a cell comprising a synthetic genotype, wherein the cell may comprise a 18 nt deletion, a 11 nt deletion, a 4 nt deletion, a 1 nt deletion, a 13 nt deletion, a substitution from G to A at the -117, of the human *HBG1* gene, *HBG2* gene, or a combination thereof.

[0080] In one example, there is described a composition, comprising a population of cells generated by a method of altering a cell disclosed herein, wherein the cells comprise a higher frequency of an alteration of a sequence of a CCAAT box target region of the human *HBG1* gene, *HBG2* gene, or a combination thereof relative to an unmodified population of cells. In certain examples, the higher frequency is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% higher. In certain examples, the alteration comprises a 18 nt deletion, a 11 nt deletion, a 4 nt deletion, a 1 nt deletion, a 13 nt deletion, a substitution from G to A at the -117, of the human *HBG1* gene, *HBG2* gene, or a combination thereof. In certain examples, at least a portion of the population of cells are within an erythroid lineage.

CLAIMS

1. A first population of modified cells comprising

a plurality of modified CD34+ or hematopoietic stem cells,

one or more of the plurality of modified cells having an indel in an *HBG* gene promoter,

the plurality of modified cells as a whole comprising the following deletions:

a HBG1/2 c.-104 to -121 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-104 to -121 deletion making up 1% to 15.5% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-110 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c. -110 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-112 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-112 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-113 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-113 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-111 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-111 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-111 to -117 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-111 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-102 to -114 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-102 to -114 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-114 to -118 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-114 to -118 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-112 to -116 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-112 to -116 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; and

a HBG1/2 c.-113 to -117 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-113 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole.

2. The first population of modified cells of claim 1, wherein the HBG1/2 c.-104 to -121 deletion makes up less than 25% of the indels in the plurality of modified cells as a whole.

3. A first population of modified cells comprising

a plurality of modified CD34+ or hematopoietic stem cells,

one or more of the plurality of modified cells including an indel in an *HBG* gene promoter, and

60% or more of the indels in the plurality of modified cells as a whole are deletions of at least 4 base pairs

the plurality of modified cells as a whole comprising the following deletions:

a HBG1/2 c.-104 to -121 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-104 to -121 deletion making up 1% to 15.5% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-110 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c. -110 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-112 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-112 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-113 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-113 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-111 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-111 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-111 to -117 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-111 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-102 to -114 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-102 to -114 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-114 to -118 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-114 to -118 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-112 to -116 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-112 to -116 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; and

a HBG1/2 c.-113 to -117 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-113 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole.

4. The first population of modified cells of any of claims 1-3, produced by a process comprising delivering a first RNP complex including a first guide RNA (gRNA) and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a first population of unmodified cells comprising a plurality of unmodified CD34+ or hematopoietic stem cells to generate the indels, the first gRNA including a first gRNA targeting domain.

5. A first population of modified cells comprising:

a plurality of modified CD34+ or hematopoietic stem cells,

one or more of the plurality of modified cells including an indel in an *HBG* gene promoter, and

60% or more of the indels in the plurality of modified cells as a whole are deletions of at least 4 base pairs;

the indels generated by delivering a first RNP complex including a first gRNA and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a first population of unmodified cells comprising a plurality of unmodified CD34+ or hematopoietic stem cells, the first gRNA including a first gRNA targeting domain,

the plurality of modified cells as a whole comprising the following deletions:

a HBG1/2 c.-104 to -121 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-104 to -121 deletion making up 1% to 15.5% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-110 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c. -110 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-112 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-112 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-113 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-113 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-111 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-111 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-111 to -117 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-111 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-102 to -114 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-102 to -114 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-114 to -118 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-114 to -118 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-112 to -116 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-112 to -116 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; and

a HBG1/2 c.-113 to -117 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-113 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole.

6. The first population of modified cells of any of claims 1-5, wherein 25% or more of the indels in the plurality of modified cells as a whole are deletions of at least 4 base pairs and are introduced by a repair mechanism other than microhomology-mediated end joining (MMEJ) repair.

7. The first population of modified cells of any of claims 1-5, wherein 25% or more of the indels in the plurality of modified cells as a whole are deletions of at least 4 base pairs and are introduced by non-homologous end joining (NHEJ) repair.

8. The first population of modified cells of any of claims 1-7, wherein 50% or more of the indels in the plurality of modified cells as a whole are deletions between 1 base pair and 25 base pairs.

9. The first population of modified cells of any of claims 1-7, wherein 50% or more of the indels in the plurality of modified cells as a whole are deletions between 3 base pairs and 25 base pairs.

10. The first population of modified cells of any of claims 1-7, wherein 50% or more of the indels in the plurality of modified cells as a whole are deletions between 4 base pairs and 25 base pairs.

11. The first population of modified cells of any of claims 1-7, wherein 50% or more of the indels in the plurality of modified cells as a whole are deletions between 5 base pairs and 25 base pairs.

12. The first population of modified cells of any of claims 1-11, wherein the plurality of modified cells as a whole comprise the following deletions:

a HBG1/2 c.-104 to -121 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-104 to -121 deletion making up 2% or more of the indels in the plurality of modified cells as a whole; and

a HBG1/2 c.-110 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c. -110 to -115 deletion making up 2% or more of the indels in the plurality of modified cells as a whole.

13. The first population of modified cells of any of claims 1-12, wherein the plurality of modified cells as a whole comprise the indels identified as having an Ave % in Indel of 0.1% or more in Table 25.

14. The first population of modified cells of any of claims 1-13, wherein the plurality of modified cells as a whole comprises the indels set forth in Table 25.

15. The first population of modified cells of any of claims 1-14, wherein the plurality of modified cells as a whole comprises at least 10% more deletions of at least 4 base pairs than a second population of modified cells comprising a plurality of modified CD34+ or hematopoietic stem cells,

one or more modified cells in the second population of modified cells having an indel in an *HBG* gene promoter; and

the indels of the second population of modified cells generated by delivering a second RNP complex comprising a second gRNA and a Cas9 RNA-guided nuclease to a second population of unmodified cells comprising a plurality of unmodified CD34+ or hematopoietic stem cells, the second gRNA including a second gRNA targeting domain comprising SEQ ID NO:339.

16. The first population of modified cells of any of claims 1-15, wherein the plurality of modified cells as a whole comprises at least 10% more deletions of at least 4 base pairs introduced by an NHEJ repair mechanism than a second population of modified cells comprising a plurality of modified CD34+ or hematopoietic stem cells,

one or more modified cells in the second population of modified cells comprising an indel in an *HBG* gene promoter; and

the indels of the second population of modified cells generated by delivering a second RNP complex comprising a second gRNA and a Cas9 RNA-guided nuclease to a second population of unmodified cells comprising a plurality of unmodified CD34+ or hematopoietic stem cells, the second gRNA including a second gRNA targeting domain comprising SEQ ID NO:339.

17. The first population of modified cells of claims 4-16, wherein the first RNP complex is delivered to the first population of unmodified cells by electroporation.

18. The first population of modified cells of claim 15 or 16, wherein the second RNP complex is delivered to the second population of unmodified cells by electroporation.

19. The first population of modified cells of any of claims 1-18, wherein the first population of unmodified cells is from a subject having sickle cell disease.

20. The first population of modified cells of claim 15 or 16, wherein the second population of unmodified cells is from a subject having sickle cell disease.

21. The first population of modified cells of any of claims 4-20, wherein the first population of modified cells comprises higher HbF levels than the first population of the unmodified cells.

22. The first population of modified cells of claim 15 or 16, wherein the first population of modified cells comprises higher HbF levels than the second population of modified cells.

23. The first population of modified cells of any of claims 4-22, the first gRNA including a 5’ end and a 3’ end, a DNA extension at the 5’ end, and a 2’-O-methyl modification and a phosphorothioate modification at the 3’ end.

24. The first population of modified cells of claim 23, wherein the DNA extension comprises a sequence set forth in SEQ ID NOs:1235-1250.

25. The first population of modified cells of any of claims 4-24, wherein the first gRNA targeting domain comprises SEQ ID NO:1254.

26. The first population of modified cells of any of claims 4-25, wherein the gRNA comprises SEQ ID NO:1051.

27. The first population of modified cells of any of claims 4-26, wherein the modified Cpf1 comprises SEQ ID NO:1097.

28. The first population of modified cells of any of claims 1-27, wherein the indel in an *HBG* gene promoter is in a CCAAT box target region.

29. An *ex vivo* or *in vitro* method of inducing expression of fetal hemoglobin (HbF) in a first population of modified cells comprising a plurality of modified CD34+ or hematopoietic stem cells, the method comprising:

delivering a first RNP complex including a first guide RNA (gRNA) and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a first population of unmodified cells comprising a plurality of unmodified CD34+ or hematopoietic stem cells to generate indels, the first gRNA including a first gRNA targeting domain,

each modified CD34+ or hematopoietic stem cell comprising an indel in an *HBG* gene promoter,

60% or more of the indels in the plurality of modified cells as a whole are deletions of at least 4 base pairs;

the first population of modified cells comprising higher HbF levels than the first population of unmodified cells,

the plurality of modified cells as a whole comprising the following deletions:

a HBG1/2 c.-104 to -121 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-104 to -121 deletion making up 1% to 15.5% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-110 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c. -110 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-112 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-112 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-113 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-113 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-111 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-111 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-111 to -117 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-111 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-102 to -114 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-102 to -114 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-114 to -118 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-114 to -118 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-112 to -116 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-112 to -116 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; and

a HBG1/2 c.-113 to -117 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-113 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole.

30. The method of inducing expression of HbF of claim 29, wherein the first population of modified cells comprises the first population of modified cells of any of claims 1-28.

31. A method of decreasing sickling in a first population of red blood cells (RBCs) cultured from a first population of modified cells comprising a plurality of modified CD34+ cells from a subject having sickle cell disease, the method comprising:

a) delivering a first RNP complex including a first gRNA and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a first population of unmodified cells comprising a plurality of unmodified CD34+ cells obtained from the subject to generate indels, the first gRNA including a first gRNA targeting domain, each modified CD34+ cell comprising an indel in an *HBG* gene promoter, 60% or more of the indels in the plurality of modified CD34+ cells as a whole are deletions of at least 4 base pairs; and

b) culturing the first population of RBCs from the first population of cells comprising the plurality of modified CD34+ cells,

the first population of RBCs exhibiting significantly decreased sickling upon deoxygenation than a second population of RBCs comprising a plurality of RBCs cultured from the first population of unmodified cells ,

the plurality of modified cells as a whole comprising the following deletions:

a HBG1/2 c.-104 to -121 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-104 to -121 deletion making up 1% to 15.5% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-110 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c. -110 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-112 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-112 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-113 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-113 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-111 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-111 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-111 to -117 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-111 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-102 to -114 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-102 to -114 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-114 to -118 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-114 to -118 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-112 to -116 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-112 to -116 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; and

a HBG1/2 c.-113 to -117 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-113 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole.

32. The method of decreasing sickling of claim 31, wherein the first population of RBCs sickle at a significantly lower oxygen tension than the second population of RBCs.

33. The method of decreasing sickling of claim 32, wherein the oxygen tension is measured by relative oxygen pressure.

34. The method of decreasing sickling of any of claims 31-33, wherein the first population of RBCs has a significantly higher minimum elongation index upon deoxygenation than the second population of RBCs.

35. The method of decreasing sickling of any of claims 31-33, wherein the first population of RBCs have a significantly higher velocity upon deoxygenation than a second population of RBCs comprising a plurality of RBCs cultured from the first population of unmodified cells.

36. The method of decreasing sickling of any of claims 31-33, wherein the first population of RBCs comprises higher HbF levels than a second population of RBCs comprising a plurality of RBCs cultured from the first population of unmodified cells.

37. The method of decreasing sickling of any of claims 31-36, wherein the first population of modified cells comprises the first population of modified cells of any of claims 1-28.

38. Use of a first population of modified cells comprising a plurality of modified CD34+ or hematopoietic stem cells in the manufacture of a medicament for alleviating one or more symptoms of sickle cell disease in a subject in need thereof, wherein the manufacture of the medicament comprises

delivering a first RNP complex including a first gRNA and a Cpf1 RNA-guided nuclease or a modified Cpf1 RNA-guided nuclease to a first population of unmodified cells comprising a plurality of unmodified CD34+ or hematopoietic stem cells to generate indels, each modified CD34+ or hematopoietic stem comprising an indel in an *HBG* gene promoter, 60% or more of the indels in a plurality of the modified CD34+ or hematopoietic stem cells as a whole are deletions of at least 4 base pairs,

the plurality of modified cells as a whole comprising the following deletions:

a HBG1/2 c.-104 to -121 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-104 to -121 deletion making up 1% to 15.5% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-110 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c. -110 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-112 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-112 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-113 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-113 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-111 to -115 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-111 to -115 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-111 to -117 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-111 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-102 to -114 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-102 to -114 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-114 to -118 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-114 to -118 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole;

a HBG1/2 c.-112 to -116 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-112 to -116 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole; and

a HBG1/2 c.-113 to -117 deletion in a *HBG1* promoter, *HBG2* promoter, or a combination thereof, the HBG1/2 c.-113 to -117 deletion making up 1% to 6% of the indels in the plurality of modified cells as a whole.

39. The use of claim 38, further comprising detecting a population of modified erythroid progeny cells comprising a plurality of modified erythroid progeny cells cultured from the first population of modified cells at one or more periods between 1 week and 5 years after administration.

40. The use of claim 38 or 39, wherein the use results in long-term engraftment of a plurality of HSC clones in bone marrow.

41. The use of any of claim 38-40, wherein the use results in long-term expression of at least 50% of total hemoglobin as compared to a healthy subject.

42. The use of any of claims 38-41, wherein the first population of modified cells comprises the first population of modified cells of any of claims 1-28.