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 including a HBG1/2 c.-104 to -121 deletion in a *HBG1* promoter, a *HBG2* promoter, or a combination thereof, and

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.

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.

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.

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 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.

14. The first population of modified cells of any of claims 1-13, wherein the plurality of modified cells as a whole comprises 108 deletions present in all 14 samples set forth in Fig. 56A.

15. The first population of modified cells of any of claims 1-13, 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.

16. The first population of modified cells of any of claims 1-15, wherein the plurality of modified cells as a whole comprises the indels set forth in Table 25.

17. The first population of modified cells of any of claims 1-16, 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.

18. The first population of modified cells of any of claims 1-17, 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.

19. The first population of modified cells of claims 4-18, wherein the first RNP complex is delivered to the first population of unmodified cells by electroporation.

20. The first population of modified cells of claim 17 or 18, wherein the second RNP complex is delivered to the second population of unmodified cells by electroporation.

21. The first population of modified cells of any of claims 1-20, wherein the first population of unmodified cells is from a subject having sickle cell disease.

22. The first population of modified cells of claim 17 or 18, wherein the second population of unmodified cells is from a subject having sickle cell disease.

23. The first population of modified cells of any of claims 4-22, wherein the first population of modified cells comprises higher HbF levels than the first population of the unmodified cells.

24. The first population of modified cells of claim 17 or 18, wherein the first population of modified cells comprises higher HbF levels than the second population of modified cells.

25. The first population of modified cells of any of claims 4-24, the first gRNA including a 5' end and a 3' end, a DNA extension at the 5' end, and ~~and~~ a 2'-O-methyl modification and a ~~3'~~ phosphorothioate modification at the 3' end.

26. The first population of modified cells of claim 25, wherein the DNA extension comprises a sequence set forth in SEQ ID NOs:1235-1250.

27. The first population of modified cells of any of claims 4-26, wherein the first gRNA targeting domain comprises SEQ ID NO:1254.

28. The first population of modified cells of any of claims 4-27, wherein the gRNA comprises SEQ ID NO:1051.

29. The first population of modified cells of any of claims 4-28, wherein the modified Cpf1 comprises SEQ ID NO:1097.

30. The first population of modified cells of any of claims 1-29, wherein the indel in an *HBG* gene promoter is in a CCAAT box target region.

31. 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.

32. The method of inducing expression of HbF of claim 31, wherein the first population of modified cells comprises the first population of modified cells of any of claims 1-30.

33. 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.

34. The method of decreasing sickling of claim 33, wherein the first population of RBCs sickle at a significantly lower oxygen tension than the second population of RBCs.

35. The method of decreasing sickling of claim 34, wherein the oxygen tension is measured by relative oxygen pressure.

36. The method of decreasing sickling of any of claims 33-35, wherein the first population of RBCs has a significantly higher minimum elongation index upon deoxygenation than the second population of RBCs.

37. The method of decreasing sickling of any of claims 33-35, 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.

38. The method of decreasing sickling of any of claims 33-35, 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.

39. The method of decreasing sickling of any of claims 33-38, wherein the first population of modified cells comprises the first population of modified cells of any of claims 1-30.

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

~~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+ 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~~; and~~

~~b) administering a first population of modified cells comprising the plurality of modified CD34+ or hematopoietic stem cells to the subject to alleviate one or more symptoms of sickle cell disease~~.

41. The method of claim 40, 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.

42. The method of claim 40 or 41, wherein the method results in long-term engraftment of a plurality of HSC clones in bone marrow.

43. The method of any of claim 40-42, wherein the method results in long-term expression of at least 50% of total hemoglobin as compared to a healthy subject.

44. The method of any of claims 40-43, wherein the first population of modified cells comprises the first population of modified cells of any of claims 1-30.