CLAIMS

1. Use of nucleobase-edited hematopoietic stem cells or progenitors thereof in the preparation of a medicament for treating a hemoglobinopathy in a subject, wherein the nucleobase-edited hematopoietic stem cells or progenitors thereof are produced by a method comprising:

(a) contacting hematopoietic stem cells or progenitors thereof *in vitro* with a guide RNA and a base editor comprising a polynucleotide programmable DNA binding domain and an adenosine deaminase domain, or a polynucleotide encoding the base editor, wherein the adenosine deaminase domain comprises an amino acid sequence that is at least 85% identical to the following amino acid sequence:
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALR
QGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNH
RVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 3), wherein the adenosine deaminase domain further comprises the amino acid alterations Y123H, Y147R, and Q154R relative to SEQ ID NO: 3, and wherein the guide RNA targets the polynucleotide programmable DNA binding domain to induce a nucleobase change in the promoter region of *HBG1/2*, thereby obtaining nucleobase-edited hematopoietic stem cells or progenitors thereof; wherein

(b) the nucleobase-edited hematopoietic stem cells or progenitors thereof are to be administered to a subject in an effective amount to obtain engraftment of the nucleobase-edited hematopoietic stem cells or progenitors thereof in tissues of the subject, and

wherein the nucleobase-edited hematopoietic stem cells have increased engraftment efficiency compared to reference nucleobase-edited hematopoietic stem cells prepared as described in (a) but with the adenosine deaminase domain comprising the amino acid sequence of SEQ ID NO: 3 with no amino acid alterations.

2. The use of claim 1, wherein the adenosine deaminase domain further comprises the alteration I76Y; and/or

wherein the adenosine deaminase domain comprises a combination of alterations selected from the group consisting of:

I76Y, Y147R, Q154R, and Y123H;

V82S, Y147R, Q154R, and Y123H; and

I76Y, V82S, Y147R, Q154R, and Y123H.

3. The use of any one of claims 1-2, wherein the deaminase domain is a TadA*8 variant; wherein the deaminase domain is a TadA*8 variant selected from the group consisting of: TadA*8.8, TadA*8.13, TadA*8.19, and TadA*8.20; and/or wherein the base editor is an ABE8 base editor selected from the group consisting of: ABE8.8, ABE8.13, ABE8.19, and ABE8.20.

4. Use of nucleobase-edited hematopoietic stem cells or progenitors thereof in the preparation of a medicament for treating a hemoglobinopathy in a subject, wherein the nucleobase-edited hematopoietic stem cells or progenitors thereof are produced by a method comprising:

(a) contacting hematopoietic stem cells or progenitors thereof *in vitro* with a guide RNA and an adenosine base editor comprising a polynucleotide programmable DNA binding domain and an adenosine deaminase domain comprising an amino acid sequence with at least 85% sequence identity to the sequence MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 3) and comprising the alterations Y123H, Y147R, and Q154R, or a polynucleotide encoding the base editor, wherein the adenosine deaminase domain catalyzes the hydrolytic deamination of adenine or adenosine, and wherein said guide RNA targets said polynucleotide programmable DNA binding domain to induce an A to G nucleobase change in the promoter region of *HBG1/*2, thereby obtaining nucleobase-edited hematopoietic stem cells or progenitors thereof;

(b) wherein the nucleobase-edited hematopoietic stem cells or progenitors thereof are to be administered to a subject in an effective amount to obtain engraftment of the nucleobase-edited hematopoietic stem cells or progenitors thereof in tissues of the subject, and

wherein the nucleobase-edited hematopoietic stem cells have increased engraftment efficiency compared to reference nucleobase-edited hematopoietic stem cells prepared as described in (a) but with the adenosine deaminase domain comprising the amino acid sequence of SEQ ID NO: 3 with no amino acid alterations.

5. The use of any one of claims 1-4, wherein the adenosine deaminase domain comprises an alteration at position 82 or 166.

6. The use of claim 5, wherein the alteration at position 82 is V82S and the alteration at position 166 is T166R.

7. The use of any one of claims 1-6, wherein the polynucleotide programmable DNA binding domain is a Cas9; or
wherein the polynucleotide programmable DNA binding domain is a Cas9 selected from SpCas9, a SaCas9, or a variant thereof.

8. The use of any one of claims 1-7, wherein the polynucleotide programmable DNA binding domain is nuclease inactive or is a nickase.

9. The the use of any one of claims 1-8, wherein the deaminase domain comprises an adenosine deaminase monomer or wherein the deaminase domain comprises an adenosine deaminase dimer.

10. Use of nucleobase-edited hematopoietic stem cells or progenitors thereof in the preparation of a medicament for treating a hemoglobinopathy in a subject, wherein the nucleobase-edited hematopoietic stem cells or progenitors thereof are produced by a method comprising:

(a) contacting hematopoietic stem cells or progenitors thereof *in vitro* with a guide RNA and a base editor comprising an amino acid sequence with at least 80% sequence identity to one of the following two amino acid sequences:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH
AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG
SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTDSGGSSG
GSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVL
GNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVD
DSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLI
YLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSA
RLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDD
LDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTL
LKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLN
REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLA
RGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY

EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIE
CFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEER
LKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNF
MQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMG
RHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLY
LYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV
PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITK
HVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDA
YLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFF
KTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS
KESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN
ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILAD
ANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLD
ATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV (SEQ ID NO: 258),
and
MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAH
AEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAA
GSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTDSGGSSG
GSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRARDEREVPVGAVL
VLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAG
AMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLCRFFR
MPRRVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIG
TNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRR
YTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEK
YPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQT
YNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNF
KSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEI
TKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEE
FYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPF
LKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFI
ERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNE
ENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI

RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAG
SPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGI
KELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFL
KDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAE
RGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVS
DFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMI
AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATV
RKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYS
VLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYS
LFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVE
QHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAP
AAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSEF
ESPKKKRKV (SEQ ID NO: 259), or a polynucleotide encoding the base editor, wherein said guide RNA targets said polynucleotide programmable DNA binding domain to induce an A to G nucleobase change in the promoter region of HBG1/2, thereby obtaining nucleobase-edited hematopoietic stem cells or progenitors thereof;

wherein each base editor comprises an adenosine deaminase domain comprising an amino acid sequence that is at least 85% identical to the following amino acid sequence: MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALR QGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNH RVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 3), wherein the adenosine deaminase domain further comprises the amino acid alterations Y123H, Y147R, and Q154R relative to SEQ ID NO: 3;

(b) wherein the nucleobase-edited hematopoietic stem cells or progenitors thereof are to be administered to a subject in an effective amount to obtain engraftment of the nucleobase-edited hematopoietic stem cells or progenitors thereof in tissues of the subject,

wherein the nucleobase-edited hematopoietic stem cells have increased engraftment efficiency compared to reference nucleobase-edited hematopoietic stem cells prepared as described in (a) but with the adenosine deaminase domain comprising the amino acid sequence of SEQ ID NO: 3 with no amino acid alterations, and a Cas9 domain.

11. The use of any one of claims 1-10, wherein the hematopoietic stem cells or progenitors thereof comprise a single-nucleotide polymorphism (SNP) associated with sickle cell disease (SCD).

12. The use of claim 11, wherein the SNP associated with SCD results in a E6V substitution in a hemoglobin beta unit encoded by the HBB gene.

13. The use of any one of claims 1-12, wherein the polynucleotide encoding the base editor comprises mRNA or is mRNA.

14. The use of any one of claims 1-13, wherein levels of fetal hemoglobin (HbF) are increased in the subject following engraftment relative to the levels in a control subject that received unedited hematopoietic stem cells or progenitors thereof.

15. The use of any one of claims 1-14, wherein the nucleobase-edited hematopoietic stem cells or progenitors thereof express one or more of CD34, CD45, CD19, and GlyA; and/or express HbF.

16. The use of any one of claims 1-15, wherein the hemoglobinopathy is sickle cell disease (SCD), thalassemia, and/or anemia.

17. The use of any one of claims 1-16, wherein the nucleobase change abolishes, disrupts, or reduces BCL11A binding in the promoter region of *HBG1/2*.

18. The use of any one of claims 1-17, wherein the nucleobase change is at a position selected from -114, -117, -175, and -198 in the promoter region of *HBG1/2*;
wherein the nucleobase change is associated with an increase in expression of HBG1/2;
wherein the nucleobase change is associated with an increase in levels of hemoglobin gamma subunit in the hematopoietic stem cells or progenitors thereof; and/or
wherein an increased level of HbF protein is expressed in the subject after administration.

19. The use of any one of claims 1-18, wherein the guide RNA comprises a nucleotide sequence selected from SEQ ID NOs: 130-155 listed in Table 1.

20. The use of any one of claims 1-18, wherein the gRNA comprises or consists of the sequence, from 5'-3':
CUUGACCAAUAGCCUUGACAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGG

CUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 129); or

from 5'-3':csususGACCAAUAGCCUUGACAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu (SEQ ID NO: 129), wherein lowercase characters indicate 2'-O-methylated nucleobases, and "s" indicates phosphorothioates (SEQ ID NO: 129).

21. The use of any one of claims 1-20, wherein the nucleobase-edited hematopoietic stem cells or progenitors thereof is contained in a kit comprising the guide RNA and a polynucleotide encoding the base editor.