CLAIMS

1. A method for editing a beta globin (*HBB*) polynucleotide associated with sickle cell disease, the method comprising contacting a cell comprising a single nucleotide polymorphism (SNP) in the beta globin (*HBB*) polynucleotide with a guide RNA and a fusion protein comprising a polynucleotide programmable DNA binding domain and an adenosine deaminase domain comprising a serine (S) at amino acid position 82 of the following amino acid sequence and having at least 85% sequence identity to the following amino acid sequence
TadA*7.10
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEI
MALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMD
VLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 2), wherein said guide RNA targets said polynucleotide programmable DNA binding domain to the SNP in the beta globin (*HBB*) polynucleotide.

2. The method of claim 1, wherein the adenosine deaminase domain further comprises an alteration at amino acid position 166.

3. The method of claim 2, wherein the alteration at amino acid position 166 is T166R.

4. The method of any one of claims 1-3, wherein the adenosine deaminase domain further comprises one or more alterations selected from the group consisting of: Y147T, Y147R, Q154S, Y123H, and Q154R.

5. The method of any one of claims 1 to 4, wherein the adenosine deaminase domain further comprises an alteration or combination of alterations selected from the group consisting of:

Y147T and Q154R;

Y147T and Q154S;

Y147R and Q154S;

Q154S;

Y147R;

Q154R;
Y123H;
I76Y;
Y123H and Y147T;
Y123H and Y147R;
Y123H and Q154R;
Y147R, Q154R, and Y123H;
Y147R, Q154R, and I76Y;
Y147R, Q154R, and T166R;
Y123H, Y147R, Q154R, and I76Y;
Y123H, Y147R, and Q154R; and
I76Y, Y123H, Y147R, and Q154R.

6. The method of any one of claims 1 to 5, wherein the adenosine deaminase domain comprises V82S and T166R.

7. The method of any one of claims 1-6, wherein the fusion protein comprises a heterodimer comprising the adenosine deaminase domain of any one of claims 1-6 and a wild-type adenosine deaminase domain.

8. The method of any one of claims 1-7, wherein the adenosine deaminase domain comprises Y147T and Q154S.

9. The method of any one of claims 1-8, wherein an A•T to G•C alteration at the SNP results in expression of an *HBB* polypeptide having an alanine at amino acid position 6.

10. The method of any one of claims 1-9, wherein the programmable DNA binding domain comprises a Cas9 domain.

11. The method of claim 10, wherein the Cas9 domain comprises a dead Cas9 (dCas9) or a nickase Cas9 (nCas9).

12. The method of claim 10 or claim 11, wherein the Cas9 domain is capable of programmable DNA binding and has at least 85% amino acid sequence identity to a DNA binding domain selected from the group consisting of *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), and a *Streptococcus thermophilus 1* Cas9 (St1Cas9).

13. The method of any one of claims 10-12, wherein the Cas9 domain comprises the following amino acid sequence:

SpCas9

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEA
TRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIV
DEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKL
FIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLG
LTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRV
NTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQ
EEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPF
LKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIER
MTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK
TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDIL
EDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGK
TILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQ
TVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS
DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKR
QLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINN
YHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKE
LLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNE
LALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANL

DKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIH
QSITGLYETRIDLSQLGGD (SEQ ID NO: 1).

14. The method of any one of claims 1-13, wherein the fusion protein is selected from the group consisting of ABE8.14-m, ABE8.15-m, ABE8.16-m, ABE8.17-m, ABE8.18-m, ABE8.19-m, ABE8.20-m, ABE8.22-m, ABE8.23-m, ABE8.24-m, ABE8.14-d, ABE8.15-d, ABE8.16-d, ABE8.17-d, ABE8.18-d, ABE8.19-d, ABE8.20-d, ABE8.22-d, ABE8.23-d, and ABE8.24-d.

15. The method of any one of claims 1 to 14, wherein the adenosine deaminase domain comprises a truncated TadA8 comprising a deletion of 1, 2, 3, 4, 5 ,6, 7, or 8 N-terminal or C-terminal amino acid residues relative to the full length TadA8.

16. The method of any one of claims 1-15, wherein the adenosine deaminase domain has at least 90% sequence identity to the amino acid sequence.

17. A base editing system comprising the fusion protein of any one of claims 1-16 and a guide RNA comprising a nucleic acid sequence selected from the group consisting of CUUCUCCACAGGAGUCAGAU (SEQ ID NO: 4); ACUUCUCCACAGGAGUCAGAU (SEQ ID NO: 5); and GACUUCUCCACAGGAGUCAGAU (SEQ ID NO: 6).

18. A method for editing a beta globin (*HBB*) polynucleotide associated with sickle cell disease, the method comprising contacting a cell comprising a single nucleotide polymorphism (SNP) in the beta globin (*HBB*) polynucleotide with a guide RNA and a fusion protein comprising a polynucleotide programmable DNA binding domain comprising the following amino acid sequence: CP5 (with MSP “NGC” PID and “D10A” nickase)
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFMQPTVAYSVLVVAKV
EKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKR
MLASAKFLQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQI
SEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPRAFKYFDTTIARKEY
RSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGSGGSGGSGGSGGSGGSGGMDKKYSIGL

AIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARR
RYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKY
PTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQ
LFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA
SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILE
KMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKI
LTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPN
EKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQL
KEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFED
REMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFA
NRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKL
YLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVP
SEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNA
VVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEGADKRTADGSEFESPKKKRKV
(SEQ ID NO: 3), and at an adenosine deaminase domain comprising a serine (S) at amino acid position 82 of the following amino acid sequence and having at least 85% sequence identity to the following amino acid sequence

TadA*7.10

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEI
MALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMD
VLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 2).

19. A cell or a progenitor thereof, comprising:

a fusion protein comprising a polynucleotide programmable DNA binding domain and an adenosine deaminase domain described in claim 1 or a polynucleotide encoding said fusion protein; and

one or more guide polynucleotides that target the fusion protein to effect an A•T to G•C alteration of the SNP in the *HBB* polynucleotide.

20. The cell of claim 19, wherein the cell is selected from the group consisting of a hematopoietic stem cell, a common myeloid progenitor, proerythroblast, erythroblast, reticulocyte, and erythrocyte.

21. The cell of claim 19 or claim 20, wherein the cell is a mammalian cell or a human cell.

22. Use of a cell comprising:

a fusion protein comprising a polynucleotide programmable DNA binding domain and an adenosine deaminase domain described in claim 1 or a polynucleotide encoding said fusion protein; and

one or more guide polynucleotides that target the fusion protein to effect an A•T to G•C alteration of the SNP in the *HBB* polynucleotide,

in the preparation of a medicament for the treatment of sickle cell disease in a subject, wherein the medicament is to be administered to the subject.

23. A population of cells comprising:

a fusion protein comprising the polynucleotide programmable DNA binding domain and the adenosine deaminase domain described in any one of claims 1-18 or a polynucleotide encoding said fusion protein; and

one or more guide polynucleotides that target the fusion protein to effect an A•T to G•C alteration of the SNP in the *HBB* polynucleotide.

24. A method of producing a red blood cell, or progenitor thereof, comprising:

(a) introducing into a red blood cell progenitor comprising an *HBB* polynucleotide associated with sickle cell disease:

i) a fusion protein, or a polynucleotide encoding said fusion protein, wherein said fusion protein comprises a polynucleotide-programmable nucleotide-binding domain and an adenosine deaminase domain described in any one of claims 1-18; and

ii) one or more guide polynucleotides, wherein said one or more guide polynucleotides target said fusion protein to effect an A•T to G•C alteration of an SNP in the *HBB* polynucleotide; and

(b) differentiating the red blood cell progenitor into a red blood cell.

25. Use of a fusion protein comprising a polynucleotide programmable DNA binding domain and an adenosine deaminase domain comprising a serine (S) at amino acid position 82 of the following amino acid sequence and having at least 85% sequence identity to the following amino acid sequence

TadA*7.10

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEI MALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMD VLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 2), or a polynucleotide encoding the fusion protein; and a guide polynucleotide to target the fusion protein to effect an A•T to G•C alteration of a single nucleotide polymorphism (SNP) associated with sickle cell disease (SCD), in the preparation of a medicament for the treatment of SCD in a subject, wherein the medicament is to be administered to the subject, thereby treating SCD in the subject.

26. The use of claim 25, wherein the guide polynucleotide is a guide RNA.

27. The use of claim 25 or claim 26, wherein the alteration of the SNP replaces a target nucleobase with a non-wild type nucleobase, and wherein the alteration of the target nucleobase is to ameliorate symptoms of sickle cell disease.

28. A pharmaceutical composition comprising a base editing system comprising the fusion protein of any one of claims 1-18, a guide RNA, and a pharmaceutically acceptable carrier, vehicle, or excipient.

29. A base editor system comprising a fusion protein comprising a polynucleotide programmable DNA binding domain and an adenosine deaminase domain comprising a serine (S) at

amino acid position 82 of the following amino acid sequence and having at least 85% sequence identity to the following amino acid sequence

TadA*7.10

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEI
MALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMD
VLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO: 2)

and a guide RNA, wherein said guide RNA targets said fusion protein to effect an alteration of a single nucleotide polymorphism (SNP) in a beta globin (*HBB*) polynucleotide.

30. The base editor system of claim 29, wherein the programmable DNA binding domain comprises the following amino acid sequence:

CP5 (with MSP "NGC" PID and "D10A" nickase)

EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFMQPTVAYSVLVVAKV
EKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKR
MLASAKFLQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQI
SEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPRAFKYFDTTIARKEY
RSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGSGGSGGSGGSGGSGGSGGMDKKYSIGL
AIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARR
RYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKY
PTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQ
LFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA
SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILE
KMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKI
LTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPN
EKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQL
KEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFED
REMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFA
NRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKL
YLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVP

SEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNA
VVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEGADKRTADGSEFESPKKKRKV
(SEQ ID NO: 3).