Claims

What is claimed is:

1. A method for editing a B-cell lymphoma 11A (BCL11A) gene in a human cell by genome editing, the method comprising the step of:

introducing into the human cell one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more single-strand breaks (SSBs) or double-strand breaks (DSBs), within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene, that results in a permanent deletion, modulation, or inactivation of a transcriptional control sequence of the BCL11A gene.

2. The method of claim 1, wherein the transcriptional control sequence is located within a second intron of the BCL11A gene.

3. The method of claims 1 or 2, wherein the transcriptional control sequence is located within a +58 DNA hypersensitive site (DHS) of the BCL11A gene.

4. An *ex vivo* method for treating a patient with a hemoglobinopathy, the method comprising the steps of:

creating a patient specific induced pluripotent stem cell (iPSC);

editing within or near a BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene of the iPSC;

differentiating the genome-edited iPSC into a hematopoietic progenitor cell; and

implanting the hematopoietic progenitor cell into the patient.

5. The method of claim 4, wherein the creating step comprises:

isolating a somatic cell from the patient; and

introducing a set of pluripotency-associated genes into the somatic cell to induce the somatic cell to become a pluripotent stem cell.

6. The method of claim 5, wherein the somatic cell is a fibroblast.

7. The method of claim 5, wherein the set of pluripotency-associated genes is one or more of the genes selected from the group consisting of OCT4, SOX2, KLF4, Lin28, NANOG and cMYC.

8. The method of any one of claims 4-7, wherein the editing step comprises introducing into the iPSC one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more single-strand breaks (SSBs) or double-strand breaks (DSBs) within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene that results in a permanent deletion, modulation, or inactivation of a transcriptional control sequence of the BCL11A gene.

9. The method of any one of claims 4-8, wherein the differentiating step comprises one or more of the following to differentiate the genome-edited iPSC into a hematopoietic progenitor cell: treatment with a combination of small molecules, delivery of master transcription factors, delivery of mRNA encoding master transcription factors, or delivery of mRNA encoding transcription factors.

10. The method of any one of claims 4-9, wherein the implanting step comprises implanting the hematopoietic progenitor cell into the patient by transplantation, local injection, systemic infusion, or combinations thereof.

11. An *ex vivo* method for treating a patient with a hemoglobinopathy, the method comprising the steps of:

isolating a mesenchymal stem cell from the patient;

editing within or near a BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene of the mesenchymal stem cell;

differentiating the genome-edited mesenchymal stem cell into a hematopoietic progenitor cell; and

implanting the hematopoietic progenitor cell into the patient.

12. The method of claim 11, wherein the mesenchymal stem cell is isolated from the patient's bone marrow or peripheral blood.

13. The method of claim 11, wherein the isolating step comprises: aspiration of bone marrow and isolation of mesenchymal cells using density gradient centrifugation media.

14. The method of any one of claims 11-13, wherein the editing step comprises introducing into the mesenchymal stem cell one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more single-strand breaks (SSBs) or double-strand breaks (DSBs) within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene that results in a permanent deletion, modulation, or inactivation of a transcriptional control sequence of the BCL11A gene.

15. The method of any one of claims 11-14, wherein the differentiating step comprises one or more of the following to differentiate the genome-edited mesenchymal stem cell into a hematopoietic progenitor cell: treatment with a combination of small molecules, delivery of master transcription factors, delivery of mRNA encoding master transcription factors, or delivery of mRNA encoding transcription factors.

16. The method of any one of claims 11-15, wherein the implanting step comprises implanting the hematopoietic progenitor cell into the patient by transplantation, local injection, systemic infusion, or combinations thereof.

17. An *ex vivo* method for treating a patient with a hemoglobinopathy, the method comprising the steps of:

isolating a hematopoietic progenitor cell from the patient;

editing within or near a BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene of the hematopoietic progenitor cell; and

implanting the genome-edited hematopoietic progenitor cell into the patient.

18. The method of claim 17, wherein the method further comprises treating the patient with granulocyte colony stimulating factor (GCSF) prior to the isolating step.

19. The method of claim 18, wherein the treating step is performed in combination with Plerixaflor.

~~20. The method of any one of claims 17-19, wherein the isolating step comprises isolating CD34+ cells.~~

~~21. The method of any one of claims 17-20, wherein the editing step comprises introducing into the hematopoietic progenitor cell one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more single-strand breaks (SSBs) or double-strand breaks (DSBs) within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene that results in a permanent deletion, modulation, or inactivation of a transcriptional control sequence of the BCL11A gene.~~

~~22. The method of any one of claims 17-21, wherein the implanting step comprises implanting the genome-edited hematopoietic progenitor cell into the patient by transplantation, local injection, systemic infusion, or combinations thereof.~~

~~23. An *in vivo* method for treating a patient with a hemoglobinopathy, the method comprising the step of editing a BCL11A gene in a cell of the patient.~~

~~24. The method of claim 23, wherein the editing step comprises introducing into the cell one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more single-strand breaks (SSBs) or double-strand breaks (DSBs) within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene that results in a permanent deletion, modulation, or inactivation of a transcriptional control sequence of the BCL11A gene.~~

~~25. The method of any one of claims 23-24, wherein the cell is a bone marrow cell, a hematopoietic progenitor cell, or a CD34+ cell.~~

~~26. The method of any one of claims 1, 8, 14, 21 or 24, wherein the one or more DNA endonucleases is a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15,~~

~~Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease; a homolog thereof, a recombination of the naturally occurring molecule thereof, codon-optimized thereof, or modified versions thereof, and combinations thereof.~~

~~27. The method of claim 26, wherein the method comprises introducing into the cell one or more polynucleotides encoding the one or more DNA endonucleases.~~

~~28. The method of claim 26, wherein the method comprises introducing into the cell one or more ribonucleic acids (RNAs) encoding the one or more DNA endonucleases.~~

~~29. The method of any one of claims 27 or 28, wherein the one or more polynucleotides or one or more RNAs is one or more modified polynucleotides or one or more modified RNAs.~~

~~30. The method of claim 26, wherein the one or more DNA endonuclease is one or more proteins or polypeptides.~~

~~31. The method of claim 30, wherein the one or more proteins or polypeptides is flanked at the N-terminus, the C-terminus, or both the N-terminus and C-terminus by one or more nuclear localization signals (NLSs).~~

~~32. The method of claim 31, wherein the one or more proteins or polypeptides is flanked by two NLSs, one NLS located at the N-terminus and the second NLS located at the C-terminus.~~

~~33. The method of any one of claims 31-32, wherein the one or more NLSs is a SV40 NLS.~~

34. The method of any one of the preceding claims, wherein the method further comprises introducing into the cell one or more guide ribonucleic acids (gRNAs).

35. The method of claim 34, wherein the one or more gRNAs are single-molecule guide RNA (sgRNAs).

36. The method of any one of claims 34-35, wherein the one or more gRNAs or one or more sgRNAs is one or more modified gRNAs or one or more modified sgRNAs.

37. The method of claim 36, wherein the one or more modified sgRNAs comprises three 2'-O-methyl-phosphorothioate residues at or near each of its 5' and 3' ends.

38. The method of claim 37, wherein the modified sgRNA is the nucleic acid sequence of SEQ ID NO: 71,959.

39. The method of any one of claims 34-38, wherein the one or more DNA endonucleases is pre-complexed with one or more gRNAs or one or more sgRNAs to form one or more ribonucleoproteins (RNPs).

40. The method of claim 39, wherein the weight ratio of sgRNA to DNA endonuclease in the RNP is 1:1.

41. The method of claim 40, wherein the sgRNA comprises the nucleic acid sequence of SEQ ID NO: 71,959, the DNA endonuclease is a *S. pyogenes* Cas9 comprising a N-terminus SV40 NLS and a C-terminus SV40 NLS, wherein the weight ratio of sgRNA to DNA endonuclease is 1:1.

42. The method of any one of the preceding claims, wherein the method further comprises introducing into the cell a polynucleotide donor template comprising a wild-type BCL11A gene or cDNA comprising a modified transcriptional control sequence.

~~43. The method of any one of claims 1, 8, 14, 21 or 24, wherein the method further comprises introducing into the cell one guide ribonucleic acid (gRNA) and a polynucleotide donor template comprising a wild-type BCL11A gene or cDNA comprising a modified transcriptional control sequence, and wherein the one or more DNA endonucleases is one or more Cas9 or Cpf1 endonucleases that effect one single-strand break (SSB) or double-strand break (DSB), at a locus within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene, that facilitates insertion of a new sequence from the polynucleotide donor template into the chromosomal DNA at the locus that results in a permanent insertion, modulation, or inactivation of the transcriptional control sequence of the chromosomal DNA proximal to the locus, and wherein the gRNA comprises a spacer sequence that is complementary to a segment of the locus.~~

~~44. The method of claim 43, wherein proximal means nucleotides both upstream and downstream of the locus.~~

~~45. The method of any one of claims 1, 8, 14, 21 or 24, wherein the method further comprises introducing into the cell one or more guide ribonucleic acid (gRNAs) and a polynucleotide donor template comprising a wild-type BCL11A gene or cDNA comprising a modified transcriptional control sequence, and wherein the one or more DNA endonucleases is one or more Cas9 or Cpf1 endonucleases that effect or create a pair of single-strand breaks (SSBs) or double-strand breaks (DSBs), the first break at a 5' locus and the second break at a 3' locus, within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene, that facilitates insertion of a new sequence from the polynucleotide donor template into the chromosomal DNA between the 5' locus and the 3' locus that results in a permanent insertion, modulation, or inactivation of the transcriptional control sequence of the chromosomal DNA between the 5' locus and the 3' locus.~~

~~46. The method of claim 45, wherein one gRNA creates a pair of SSBs or DSBs.~~

47. The method of claim 45, wherein one gRNA comprises a spacer sequence that is complementary to either the 5’ locus or the 3’ locus.

48. The method of claim 45, wherein the method comprises a first guide RNA and a second guide RNA, wherein the first guide RNA comprises a spacer sequence that is complementary to a segment of the 5' locus and the second guide RNA comprises a spacer sequence that is complementary to a segment of the 3' locus.

49. The method of any one of claims 43-48, wherein the one or two gRNAs are one or two single-molecule guide RNA (sgRNAs).

50. The method of any one of claims 43-49, wherein the one or two gRNAs or one or two sgRNAs is one or two modified gRNAs or one or two modified sgRNAs.

51. The method of claim 50, wherein the one modified sgRNA comprises three 2’-O-methyl-phosphorothioate residues at or near each of its 5’ and 3’ ends.

52. The method of claim 51, wherein the one modified sgRNA is the nucleic acid sequence of SEQ ID NO: 71,959.

53. The method of any one of claims 43-52, wherein the one or more Cas9 endonucleases is pre-complexed with one or two gRNAs or one or two sgRNAs to form one or more ribonucleoproteins (RNPs).

54. The method of claim 53, wherein the one or more Cas9 endonuclease is flanked at the N-terminus, the C-terminus, or both the N-terminus and C-terminus by one or more nuclear localization signals (NLSs).

55. The method of claim 54, wherein the one or more Cas9 endonucleases is flanked by two NLSs, one NLS located at the N-terminus and the second NLS located at the C-terminus.

56. The method of any one of claims 54-55, wherein the one or more NLSs is a SV40 NLS.

57. The method of claim 53, wherein the weight ratio of sgRNA to Cas9 endonuclease in the RNP is 1:1.

58. The method of claim 53, wherein the one sgRNA comprises the nucleic acid sequence of SEQ ID NO: 71,959, the Cas9 endonuclease is a *S. pyogenes* Cas9 comprising a N-terminus SV40 NLS and a C-terminus SV40 NLS, wherein the weight ratio of sgRNA to Cas9 endonuclease is 1:1.

59. The method of any one of claims 43-58, wherein the donor template is either single or double stranded.

60. The method of claims 42-59, wherein the modified transcriptional control sequence is located within a second intron of the BCL11A gene.

61. The method of claims 42-59, wherein the modified transcriptional control sequence is located within a +58 DNA hypersensitive site (DHS) of the BCL11A gene.

62. The method of claims 42-61, wherein the insertion is by homology directed repair (HDR).

63. The method of claims 8, 14, 21, 24, 43, and 45, wherein the SSB, DSB, or 5' locus and 3' locus are located within a second intron of the BCL11A gene.

~~64. The method of claims 8, 14, 21, 24, 43, and 45, wherein the SSB, DSB, or 5' locus and 3' locus are located within a +58 DNA hypersensitive site (DHS) of the BCL11A gene.~~

~~65. The method of any one of claims 1, 8, 14, 21, or 24, wherein the method further comprises introducing into the cell one or more guide ribonucleic acid (gRNAs), and wherein the one or more DNA endonucleases is one or more Cas9 or Cpf1 endonucleases that effect or create a pair of single-strand breaks (SSBs) or double-strand breaks (DSBs), a first SSB or DSB at a 5' locus and a second SSB or DSB at a 3' locus, within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene that causes a deletion of the chromosomal DNA between the 5' locus and the 3' locus that results in a permanent deletion, modulation, or inactivation of the transcriptional control sequence of the chromosomal DNA between the 5' locus and the 3' locus.~~

~~66. The method of claim 65, wherein one gRNA creates a pair of SSBs or DSBs.~~

~~67. The method of claim 65, wherein one gRNA comprises a spacer sequence that is complementary to either the 5' locus or the 3' locus.~~

~~68. The method of claim 65, wherein the method comprises a first guide RNA and a second guide RNA, wherein the first guide RNA comprises a spacer sequence that is complementary to a segment of the 5' locus and the second guide RNA comprises a spacer sequence that is complementary to a segment of the 3' locus.~~

~~69. The method of claims 65-68, wherein the one or more gRNAs are one or more single-molecule guide RNA (sgRNAs).~~

~~70. The method of any one of claims 65-69, wherein the one or more gRNAs or one or more sgRNAs are one or more modified gRNAs or one or more modified sgRNAs.~~

~~71. The method of claim 70, wherein the one modified sgRNA comprises three 2'-O-methyl-phosphorothioate residues at or near each of its 5' and 3' ends.~~

~~72. The method of claim 71, wherein the one modified sgRNA is the nucleic acid sequence of SEQ ID NO: 71,959.~~

~~73. The method of any one of claims 65-72, wherein the one or more Cas9 endonucleases is pre-complexed with one or more gRNAs or one or more sgRNAs to form one or more ribonucleoproteins (RNPs).~~

~~74. The method of claim 73, wherein the one or more Cas9 endonuclease is flanked at the N-terminus, the C-terminus, or both the N-terminus and C-terminus by one or more nuclear localization signals (NLSs).~~

~~75. The method of claim 74, wherein the one or more Cas9 endonucleases is flanked by two NLSs, one NLS located at the N-terminus and the second NLS located at the C-terminus.~~

~~76. The method of any one of claims 74-75, wherein the one or more NLSs is a SV40 NLS.~~

~~77. The method of claim 73, wherein the weight ratio of sgRNA to Cas9 endonuclease in the RNP is 1:1.~~

78. The method of claim 73, wherein the one sgRNA comprises the nucleic acid sequence of SEQ ID NO: 71,959, the Cas9 endonuclease is a *S. pyogenes* Cas9 comprising a N-terminus SV40 NLS and a C-terminus SV40 NLS, wherein the weight ratio of sgRNA to Cas9 endonuclease is 1:1.

79. The method of any one of claims 65-78, wherein both the 5' locus and 3' locus are located within a second intron of the BCL11A gene.

80. The method of any one of claims 65-78, wherein both the 5' locus and 3' locus are located within a +58 DNA hypersensitive site (DHS) of the BCL11A gene.

81. The method of any one of claims 1, 8, 14, 21, or 24-80, wherein the Cas9 or Cpf1 mRNA, gRNA, and donor template are either each formulated into separate lipid nanoparticles or all co-formulated into a lipid nanoparticle.

82. The method of any one of claims 1, 8, 14, 21, or 24-80, wherein the Cas9 or Cpf1 mRNA is formulated into a lipid nanoparticle, and both the gRNA and donor template are delivered to the cell by an adeno-associated virus (AAV) vector.

83. The method of any one of claims 1, 8, 14, 21, or 24-80, wherein the Cas9 or Cpf1 mRNA is formulated into a lipid nanoparticle, and the gRNA is delivered to the cell by electroporation and donor template is delivered to the cell by an adeno-associated virus (AAV) vector.

84. The method of any one of claims 1, 8, 14, 21, or 24-80, wherein the one or more RNP is delivered to the cell by electroporation.

85. The method of any one of the preceding claims, wherein the BCL11A gene is located on Chromosome 2: 60,451,167 – 60,553,567 (Genome Reference Consortium – GRCh38).

~~86. The method of any one of the preceding claims, wherein the hemoglobinopathy is selected from a group consisting of sickle cell anemia and thalassemia (α, β, δ, γ, and combinations thereof)~~

~~87. The method of any preceding claim, wherein the editing the BCL11A gene reduces BCL11A gene expression.~~

~~88. One or more guide ribonucleic acids (gRNAs) for editing a BCL11A gene in a cell from a patient with a hemoglobinopathy, the one or more gRNAs comprising a spacer sequence selected from the group consisting of nucleic acid sequences in SEQ ID NOs: 1 - 71,947 of the Sequence Listing.~~

~~89. The one or more gRNAs of claim 88, wherein the one or more gRNAs are one or more single-molecule guide RNAs (sgRNAs).~~

~~90. The one or more gRNAs or sgRNAs of claims 88 or 89, wherein the one or more gRNAs or one or more sgRNAs is one or more modified gRNAs or one or more modified sgRNAs.~~

~~91. The one or more modified sgRNAs of claim 90, wherein the one or more modified sgRNAs comprises three 2'-O-methyl-phosphorothioate residues at or near each of its 5' and 3' ends.~~

~~92. The one or more modified sgRNAs of claim 91, wherein the one or more modified sgRNAs comprises the nucleic acid sequence of SEQ ID NO: 71,959.~~

~~93. A single-molecule guide RNA (sgRNA) comprising the nucleic acid sequence of SEQ ID NO: 71,959.~~

1. A *ex vivo* method for editing a B-cell lymphoma 11A (BCL11A) gene in a human cell by genome editing, the method comprising the step of:

introducing into the human cell *ex vivo* one or more deoxyribonucleic acid (DNA) endonucleases and a single-molecule guide ribonucleic acid (sgRNA) comprising the nucleic acid sequence of SEQ ID NO: 71,959 to effect one or more single-strand breaks (SSBs) or double-strand breaks (DSBs), within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene, that results in a permanent deletion, modulation, or inactivation of a transcriptional control sequence of the BCL11A gene.

2. An *ex vivo* method, the method comprising the steps of:
creating a subject specific induced pluripotent stem cell (iPSC) or obtaining a mesenchymal stem cell isolated from the subject;
editing within or near a BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene of the iPSC or the mesenchymal stem cell by introducing one or more deoxyribonucleic acid (DNA) endonucleases and a single-molecule guide ribonucleic acid (sgRNA) comprising the nucleic acid sequence of SEQ ID NO: 71,959;
differentiating the genome-edited iPSC or the mesenchymal stem cell into a hematopoietic progenitor cell; and
recovering the hematopoietic progenitor cell for implanting into the subject.

3. The method of claim 2, wherein:
the creating step comprises recovering a somatic cell isolated from the subject, or the isolated cell is isolated by aspiration of bone marrow and isolation of mesenchymal cells using density gradient centrifugation media; and
introducing a set of pluripotency-associated genes into the somatic cell to induce the somatic cell to become a pluripotent stem cell.

4. The method of claim 3, wherein the set of pluripotency-associated genes is one or more of the genes selected from the group consisting of OCT4, SOX2, KLF4, Lin28, NANOG and cMYC.

5. The method of claim 2, wherein the method meets any one or more of the following:

(i) the differentiating step comprises one or more of the following to differentiate the genome-edited iPSC or the mesenchymal stem cell into a hematopoietic progenitor cell: treatment with a combination of small molecules, delivery of master transcription factors, delivery of mRNA encoding master transcription factors, or delivery of mRNA encoding transcription factors; or

(ii) the recovered cell is for being implanted into the patient by transplantation, local injection, systemic infusion, or combinations thereof.

6. An *ex vivo* method, the method comprising the steps of:

obtaining a hematopoietic progenitor cell isolated from a subject;

editing within or near a BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene of the hematopoietic progenitor cell by introducing one or more deoxyribonucleic acid (DNA) endonucleases and a single-molecule guide ribonucleic acid (sgRNA) comprising the nucleic acid sequence of SEQ ID NO: 71,959; and

recovering the genome-edited hematopoietic progenitor cell for implanting into the patient.

7. The method of any one of claims 1, 2, or 5, wherein the one or more DNA endonucleases is selected from a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease; a homolog thereof, a recombination of the naturally occurring molecule thereof, codon-optimized thereof, or modified versions thereof, and combinations thereof.

8. The method of claim 7, wherein the method comprises (i) introducing into the cell one or more polynucleotides encoding the one or more DNA endonucleases; or (ii) introducing into the cell one or more ribonucleic acids (RNAs) encoding the one or more DNA endonucleases.

9. The method of claim 7, wherein the one or more DNA endonuclease is one or more proteins or polypeptides.

10. The method of claim 9, wherein the one or more proteins or polypeptides is flanked at the N-terminus, the C-terminus, or both the N-terminus and C-terminus by one or more nuclear localization signals (NLSs).

11. The method of any one of claims 1, 2, or 5, wherein the sgRNA comprises three 2'-O-methyl-phosphorothioate residues at or near each of its 5' and 3' ends.

12. The method of any one of claims 1, 2, or 5, wherein the one or more DNA endonucleases is pre-complexed with the sgRNA to form one or more ribonucleoproteins (RNPs).

13. The method of claim 12, wherein the one or more DNA endonucleases is combined with the sgRNA in a weight ratio of 1:1 DNA endonuclease to sgRNA to form the one or more ribonucleoproteins (RNPs).

14. The method of claim 12, wherein the sgRNA comprises the nucleic acid sequence of SEQ ID NO: 71,959, the DNA endonuclease is a *S. pyogenes* Cas9 comprising a N-terminus SV40 NLS and a C-terminus SV40 NLS, wherein the one or more DNA endonucleases is combined with the sgRNA in a weight ratio of 1:1 DNA endonuclease to sgRNA to form the one or more ribonucleoproteins (RNPs).

15. The method of any one of claims 1-6, wherein the method further comprises introducing into the cell a polynucleotide donor template comprising a wild-type BCL11A gene or cDNA comprising a modified transcriptional control sequence.

16. The method of any one of claims 1, 2, or 5, wherein:
(i) the method further comprises introducing into the cell a polynucleotide donor template comprising a wild-type BCL11A gene or cDNA comprising a modified transcriptional control sequence, and wherein the one or more DNA endonucleases is one or more Cas9 or Cpf1 endonucleases that effect one single-strand break (SSB) or

double-strand break (DSB), at a locus within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene, that facilitates insertion of a new sequence from the polynucleotide donor template into the chromosomal DNA at the locus that results in a permanent insertion, modulation, or inactivation of the transcriptional control sequence of the chromosomal DNA proximal to the locus, and wherein the gRNA comprises a spacer sequence that is complementary to a segment of the locus; or

(ii) the method further comprises introducing into the cell a polynucleotide donor template comprising a wild-type BCL11A gene or cDNA comprising a modified transcriptional control sequence, and wherein the one or more DNA endonucleases is one or more Cas9 or Cpf1 endonucleases that effect or create a pair of single-strand breaks (SSBs) or double-strand breaks (DSBs), the first break at a 5' locus and the second break at a 3' locus, within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene, that facilitates insertion of a new sequence from the polynucleotide donor template into the chromosomal DNA between the 5' locus and the 3' locus that results in a permanent insertion, modulation, or inactivation of the transcriptional control sequence of the chromosomal DNA between the 5' locus and the 3' locus.

17. The method of claim 16, wherein the one or more Cas9 endonucleases is pre-complexed with the sgRNA to form one or more ribonucleoproteins (RNPs).

18. The method of claim 17, wherein the one or more Cas9 endonucleases is combined with the sgRNA in a weight ratio of 1:1 Cas9 endonuclease to sgRNA to form the one or more ribonucleoproteins (RNPs).

19. The method of claim 16, wherein

(i) the donor template is either single or double stranded; or

(ii) the modified transcriptional control sequence is located within a second intron of the BCL11A gene or the modified transcriptional control sequence is located within a +58 DNAse hypersensitive site (DHS) of the BCL11A gene.

20. The method of claim 16, wherein

(i) the Cas9 or Cpf1 are introduced by mRNA, and wherein the mRNA, sgRNA, and donor template are either each formulated into separate lipid nanoparticles or all co-formulated into a lipid nanoparticle; or

(ii) the Cas9 or Cpf1 are introduced by mRNA, and wherein the mRNA is formulated into a lipid nanoparticle, and both the sgRNA and donor template are delivered to the cell by an adeno-associated virus (AAV) vector; or

(iii) the Cas9 or Cpf1 are introduced by mRNA, and wherein the mRNA is formulated into a lipid nanoparticle, and the sgRNA is delivered to the cell by electroporation and donor template is delivered to the cell by an adeno-associated virus (AAV) vector.

21. The method of claim 12, wherein the one or more RNP is delivered to the cell by electroporation.

22. The method of claim 17, wherein the one or more RNP is delivered to the cell by electroporation.

23. A single-molecule guide RNA (sgRNA) comprising the nucleic acid sequence of SEQ ID NO: 71,959.

24. A genetically engineered cell, characterized in that is obtainable by a method comprising:

introducing into a human cell one or more S. pyogenes Cas9 endonucleases and one or more guide RNAs (gRNAs) to effect one or more double-strand breaks (DSBs) within or near a B-cell lymphoma 11A (BCL11A) gene or other DNA sequence that encodes a regulatory element of the BCL11A gene, that results in a permanent deletion, modulation, or inactivation of a transcripstional control sequence of the BCL11A gene, wherein the one or more gRNAs comprise a single-molecule guide RNA (sgRNA) of SEQ ID NO: 71,959.

25. A genetically engineered cell, characterized in that comprises a genetic mutation, which is one of a permanent deletion, modulation, or inactivation of a transcriptional control sequence of a B-cell lymphoma 11A (BCL11A) gene, wherein the

genetic mutation occurs at the site target by a gRNA comprising a single-molecule guide RNA (sgRNA) of SEQ ID NO: 71,959.

26. The sgRNA of claim 23, wherein the sgRNA comprises three 2'-O-methyl-phosphorothioate residues at or near each of its 5' and 3' ends.

27. The genetically engineered cell of claim 24 or 25, wherein the sgRNA comprises three 2'-O-methyl-phosphorothioate residues at or near each of its 5' and 3' ends.