

CLAIMS

1. An engineered cell, comprising at least one sequence encoding a transgene, wherein the at least one sequence is inserted within a safe harbor locus;

wherein the safe harbor locus is at any one or more of ~~an~~ the sgRNA target loci selected from: chr11:128340000-128350000, chr10:33130000-33140000, chr10:72290000-72300000, chr11:65425000-65427000 (NEAT1), chr15:92830000-92840000, chr16:11220000-11230000, chr2:87460000-87470000, chr3:186510000-186520000, chr3:59450000-59460000, chr8:127980000-128000000, chr9:7970000-7980000, APRT, B2M, CAPNS1, CBLB, CD2, CD3E, CD3G, CD5, EDF1, FTL, PTEN, PTPN2, PTPN6, PTPRC, PTPRCAP, RPS23, RTRAF, SERF2, SLC38A1, SMAD2, SOCS1, SRP14, SRSF9, SUB1, TET2, TIGIT, TRAC, and TRIM28, provided in Table 4; and

2. The engineered cell of claim 1, wherein expression of the at least one sequence encoding the transgene is operatively linked to an endogenous promoter.

~~3. 2. The engineered cell of claim 1, An engineered cell, comprising at least one sequence encoding a transgene, wherein the at least one sequence is inserted within a safe harbor locus, the safe harbor locus is at any one or more of an sgRNA target loci provided in Table 4; and wherein~~
expression of the at least one sequence encoding the transgene is operatively linked to an exogenous promoter.

~~4. 3. —~~ The engineered cell of claim 1 ~~or 2~~, wherein the ~~sgRNA~~ target locus is selected from: chr11:128340000-128350000, chr10:33130000-33140000, chr10:72290000-72300000, chr11:65425000-65427000 (NEAT1), chr15:92830000-92840000, chr16:11220000-11230000, chr2:87460000-87470000, chr3:186510000-186520000, chr3:59450000-59460000, chr8:127980000-128000000, ~~or and~~ chr9:7970000-7980000.

~~4. — The engineered cell of any one of claims 1-3, wherein the sgRNA target locus is selected from: chr11:128340000-128350000, chr10:72290000-72300000, chr15:92830000-92840000, or chr16:11220000-11230000.~~

5. The engineered cell of claim 1 ~~any one of claims 1-4~~, wherein the ~~sgRNA~~ target locus is chr11:128340000-128350000 or chr15:92830000-92840000.

~~6. — The engineered cell of any one of claims 1-4, wherein the sgRNA target locus is chr15:92830000-92840000.~~

~~6.~~ 7.—The engineered cell of claim 1 ~~or 2~~, wherein the ~~sgRNA~~ target locus is a gene selected from: *APRT*, *B2M*, *CAPNS1*, *CBLB*, *CD2*, *CD3E*, *CD3G*, *CD5*, *EDF1*, *FTL*, *PTEN*, *PTPN2*, *PTPN6*, *PTPRC*, *PTPRCAP*, *RPS23*, *RTRAF*, *SERF2*, *SLC38A1*, *SMAD2*, *SOCs1*, *SRP14*, *SRSF9*, *SUB1*, *TET2*, *TIGIT*, *TRAC*, ~~or and~~ TRIM28.

~~8.~~—The engineered cell of any one of claims 1-3, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: ~~GS94, GS88, GS89, GS90, GS91, GS92, GS93, GS95, GS96, GS97, GS98, GS99, GS100, GS101, GS102, GS103, GS104, GS105, GS106, GS107, GS108, GS109, GS110, GS111, GS112, GS113, GS114, GS115, GS116, GS117, GS118, GS119, or GS120.~~

~~9.~~—The engineered cell of any one of claims 1-4, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: ~~GS94, GS91, GS92, GS93, GS95, GS96, GS100, GS101, GS102, GS103, GS104, and GS105.~~

~~10.~~—The engineered cell of claim 8 or 9, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: ~~GS103, GS104, or GS105.~~

~~11.~~—The engineered cell of claim 8 or 9, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: ~~GS94, GS95, or GS96.~~

~~12.~~—The engineered cell of claim 8 or 9, wherein the safe harbor locus is an ~~GS94~~ integration site in Table 4.

~~13.~~—The engineered cell of claim 8 or 9, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: ~~GS100, GS101, or GS102.~~

7. ~~14.~~—The engineered cell of claim ~~18~~ or 9, wherein the safe harbor locus is ~~an~~ the GS94 or GS102 integration site in Table 4.

~~15.~~—The engineered cell of claim 8 or 9, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: ~~GS91, GS92, or GS93.~~

8. ~~16.~~—The engineered cell of claim 3 ~~any one of claims 2-15~~, wherein the exogenous promoter is an EF1a promoter.

9. ~~17.~~—The engineered cell of claim 1 ~~any one of claims 1-16~~, wherein the engineered cell is a natural killer (NK) cell, an induced pluripotent stem cells (iPSC), a human pluripotent stem cell (HSPC), a primary cell, an hematopoietic cell, an adaptive immune cell, an innate immune cell, a T cell or a T cell progenitor.

~~18.~~—The engineered cell of claim 17, wherein the cell is a T cell or a T cell progenitor.

- ~~19. The engineered cell of any one of claims 1-18, wherein the engineered cell is undifferentiated.~~
- ~~20. The engineered cell of any one of claims 1-18, wherein the engineered cell is CD45RA⁺ and CCR7⁺.~~
- ~~10. 21. The engineered cell of claim 1 any one of claims 1-20, wherein the transgene encodes a recombinant protein, optionally a therapeutic agent, or a chimeric antigen receptor (CAR).~~
- ~~22. The engineered cell of any one of claims 1-21, wherein the transgene encodes a chimeric antigen receptor (CAR).~~
- ~~11. 23. A composition comprising the engineered cell of claim 1 any one of claims 1-22 and a pharmaceutical excipient.~~
- ~~12. 24. A guide ribonucleic acids (gRNA) for editing a cell at a safe harbor locus, wherein the gRNA comprises any one of the sgRNA sequences in Table 4 SEQ ID NOS: 94, 102, 1-93, 95-101, or 103-120.~~
- ~~25. The gRNA of claim 24, wherein the gRNA comprises any one of SEQ ID NOS: 1-120.~~
- ~~26. The gRNA of claim 24 or 25, wherein the gRNA comprises any one of SEQ ID NOS: 91-96 and 100-105.~~
- ~~27. The gRNA of any one of claims 24-26, wherein the gRNA comprises SEQ ID NO: 94 or SEQ ID NO: 102.~~
- ~~28. The gRNA of any one of claims 24-26, wherein the gRNA comprises SEQ ID NO: 94.~~
- ~~29. The gRNA of any one of claims 24-26, wherein the gRNA comprises SEQ ID NO: 102.~~
- ~~30. The gRNA of any one of claims 24-29, wherein the cell is a stem cell, a human cell, a primary cell, an hematopoietic cell, an adaptive immune cell, an innate immune cell, a T cell or a T cell progenitor.~~
- ~~13. 31. A method of editing a cell having chromosomal DNA, comprising inserting at least one sequence encoding a transgene within a safe harbor locus in the chromosomal DNA of the cell, wherein the safe harbor locus is at any one or more of the sgRNA target loci provided in Table 4: selected from: chr11:128340000-128350000, chr10:33130000-33140000, chr10:72290000-72300000, chr11:65425000-65427000 (NEAT1), chr15:92830000-92840000, chr16:11220000-11230000, chr2:87460000-87470000, chr3:186510000-186520000, chr3:59450000-59460000, chr8:127980000-128000000, chr9:7970000-7980000, APRT, B2M,~~

CAPNS1, CBLB, CD2, CD3E, CD3G, CD5, EDF1, FTL, PTEN, PTPN2, PTPN6, PTPRC, PTPRCAP, RPS23, RTRAF, SERF2, SLC38A1, SMAD2, SOCS1, SRP14, SRSF9, SUB1, TET2, TIGIT, TRAC, and TRIM28.

14. ~~**32.**~~—The method of claim ~~13~~**31**, wherein the ~~sgRNA~~ target locus is selected from: chr11:128340000-128350000, chr10:33130000-33140000, chr10:72290000-72300000, chr11:65425000-65427000 (NEAT1), chr15:92830000-92840000, chr16:11220000-11230000, chr2:87460000-87470000, chr3:186510000-186520000, chr3:59450000-59460000, chr8:127980000-128000000, ~~and/or~~ chr9:7970000-7980000.

~~**33.**—The method of claim 31 or 32, wherein the sgRNA target locus is selected from: chr11:128340000-128350000, chr10:72290000-72300000, chr15:92830000-92840000, or chr16:11220000-11230000.~~

15. ~~**34.**~~—The method of ~~claim 13~~any one of claims 31-33, wherein the ~~sgRNA~~ target locus is chr11:128340000-128350000 or chr15:92830000-92840000.

~~**35.**—The method of any one of claims 31-33, wherein the sgRNA target locus is chr15:92830000-92840000.~~

16. ~~**36.**~~—The method of claim ~~13~~**31**, wherein the ~~sgRNA~~ target locus is a gene selected from: APRT, B2M, CAPNS1, CBLB, CD2, CD3E, CD3G, CD5, EDF1, FTL, PTEN, PTPN2, PTPN6, PTPRC, PTPRCAP, RPS23, RTRAF, SERF2, SLC38A1, SMAD2, SOCS1, SRP14, SRSF9, SUB1, TET2, TIGIT, TRAC, ~~or~~ and TRIM28.

~~**37.**—The method of claim 31 or 32, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: GS94, GS88, GS89, GS90, GS91, GS92, GS93, GS95, GS96, GS97, GS98, GS99, GS100, GS101, GS102, GS103, GS104, GS105, GS106, GS107, GS108, GS109, GS110, GS111, GS112, GS113, GS114, GS115, GS116, GS117, GS118, GS119, or GS120.~~

~~**38.**—The method of any one of claims 31-33, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: GS94, GS91, GS92, GS93, GS95, GS96, GS100, GS101, GS102, GS103, GS104, or GS105.~~

~~**39.**—The method of any one of claims 31-33, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: GS103, GS104, or GS105.~~

~~**40.**—The method of any one of claims 31-33, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: GS94, GS95, or GS96.~~

- ~~41.~~—The method of any one of claims 31–33, wherein the safe harbor locus is the GS94 integration site in Table 4.
- ~~42.~~—The method of any one of claims 31–33, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: GS100, GS101, or GS102.
- ~~43.~~—The method of any one of claims 31–33, wherein the safe harbor locus is the GS102 integration site in Table 4.
- ~~44.~~—The method of any one of claims 31–33, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: GS91, GS92, or GS93.
17. ~~45.~~—The method of claim 13~~any one of claims 31–44~~, wherein the transgene encodes a recombinant protein, ~~optionally~~ a therapeutic agent, or a chimeric antigen receptor (CAR).
- ~~46.~~—The method of any one of claims 31–45, wherein the transgene encodes a chimeric antigen receptor (CAR).
18. ~~47.~~—The method of claim 13~~any one of claims 31–46~~, wherein the at least one sequence comprises an exogenous promoter and the exogenous promoter is operably linked to the transgene.
- ~~48.~~—The method of claim 47, wherein the exogenous promoter is an EF1a promoter.
- ~~49.~~—The method of any one of claims 31–48, wherein the cell is a stem cell, a human cell, a primary cell, an hematopoietic cell, an adaptive immune cell, an innate immune cell, a T cell or T cell progenitor.
19. ~~50.~~—The method of claim 13~~49~~, wherein the cell is a T cell or ~~a~~ T cell progenitor.
- ~~51.~~—The method of any one of claims 31–50, wherein the engineered cell is undifferentiated.
- ~~52.~~—The method of any one of claims 31–51, wherein the engineered cell is CD45RA⁺ and CCR7⁺.
20. ~~53.~~—The method of claim 13~~any one of claims 31–52~~, wherein the at least one sequence is inserted using a homology-directed repair or a homology independent targeted insertion.
- ~~54.~~—The method of any one of claims 31–52, wherein the at least one sequence is inserted using a homology independent targeted insertion.
21. ~~55.~~—The method of claim 13~~any one of claims 31–54~~, wherein the at least one sequence is inserted using one or more guide ribonucleic acids (gRNAs) and one or more Cas9

endonucleases, wherein the one or more gRNAs comprises any one of SEQ ID NOS: 94, 102, 1-93, 95-101, or 103-120.

~~56. The method of claim 55, wherein the one or more gRNAs comprises any one of SEQ ID NOS: 1-120.~~

~~57. The method of claim 55 or 56, wherein the one or more gRNAs comprises any one of SEQ ID NOS: 91-96 and 100-105.~~

~~58. The method of any one of claims 55-57, wherein the gRNA comprises SEQ ID NO:94 or SEQ ID NO:102.~~

~~59. The method of any one of claims 55-58, wherein the gRNA comprises SEQ ID NO:94.~~

~~60. The method of any one of claims 55-58, wherein the gRNA comprises SEQ ID NO:102.~~

~~61. A method of editing a T cell, comprising contacting a T cell with one or more guide ribonucleic acids (gRNAs), at least one sequence encoding a transgene, and one or more Cas9 endonucleases, wherein the one or more gRNAs and Cas9 endonucleases facilitate the insertion of the at least one sequence into chromosomal DNA within a safe harbor locus, wherein the safe harbor locus is selected from any one or more of an sgRNA target loci in Table 4.~~

~~62. The method of claim 61, wherein the one or more gRNAs comprises a sequence selected from any one of the sgRNA sequences in Table 4.~~

~~63. The method of claim 61 or 62, wherein the one or more gRNAs comprises any one of SEQ ID NOS: 1-120.~~

~~64. The method of any one of claims 61-63, wherein the one or more gRNAs comprises any one of SEQ ID NOS: 91-96 and 100-105.~~

~~65. The method of any one of claims 61-64, wherein the gRNA comprises SEQ ID NO:94 or SEQ ID NO:102.~~

~~66. The method of any one of claims 61-65, wherein the gRNA comprises SEQ ID NO:94.~~

~~67. The method of any one of claims 61-65, wherein the gRNA comprises SEQ ID NO:102.~~

~~68. The method of any one of claims 61-67, wherein the sgRNA target locus is selected from: chr11:128340000-128350000, chr10:33130000-33140000, chr10:72290000-72300000, chr11:65425000-65427000 (NEAT1), chr15:92830000-92840000, chr16:11220000-11230000, chr2:87460000-87470000, chr3:186510000-186520000, chr3:59450000-59460000, chr8:127980000-128000000, or chr9:7970000-7980000.~~

- 69.**—The method of any one of claims 61–68, wherein the sgRNA target locus is selected from: ~~chr11:128340000–128350000, chr10:72290000–72300000, chr15:92830000–92840000, or chr16:11220000–11230000.~~
- 70.**—The method of any one of claims 61–69, wherein the sgRNA target locus is ~~chr11:128340000–128350000.~~
- 71.**—The method of any one of claims 61–69, wherein the sgRNA target locus is ~~chr15:92830000–92840000.~~
- 72.**—The method of claim 61–67, wherein the sgRNA target locus is a gene selected from: ~~*APRT*, *B2M*, *CAPNS1*, *CBLB*, *CD2*, *CD3E*, *CD3G*, *CD5*, *EDF1*, *FTL*, *PTEN*, *PTPN2*, *PTPN6*, *PTPRC*, *PTPRCAP*, *RPS23*, *RTRAF*, *SERF2*, *SLC38A1*, *SMAD2*, *SOCS1*, *SRP14*, *SRSF9*, *SUB1*, *TET2*, *TIGIT*, *TRAC*, or *TRIM28*.~~
- 73.**—The method of any one of claims 61–68, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: ~~GS94, GS88, GS89, GS90, GS91, GS92, GS93, GS95, GS96, GS97, GS98, GS99, GS100, GS101, GS102, GS103, GS104, GS105, GS106, GS107, GS108, GS109, GS110, GS111, GS112, GS113, GS114, GS115, GS116, GS117, GS118, GS119, or GS120.~~
- 74.**—The method of any one of claims 61–68 and 73, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: ~~GS94, GS91, GS92, GS93, GS95, GS96, GS100, GS101, GS102, GS103, GS104, or GS105.~~
- 75.**—The method of any one of claims 61–68 and 73–74, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: ~~GS103, GS104, or GS105.~~
- 76.**—The method of any one of claims 61–68 and 73–74, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: ~~GS94, GS95, and GS96.~~
- 77.**—The method of claim 76, wherein the safe harbor locus is the GS94 integration site in Table 4.
- 78.**—The method of any one of claims 61–68 and 73–74, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: ~~GS100, GS101, or GS102.~~
- 79.**—The method of claim 78, wherein the safe harbor locus is the GS102 integration site in Table 4.
- 80.**—The method of any one of claims 61–68 and 73–74, wherein the safe harbor locus is selected from any one of the integration sites in Table 4 designated: ~~GS91, GS92, or GS93.~~

~~81. The method of any one of claims 61-80, wherein the engineered cell is undifferentiated.~~

~~82. The method of any one of claims 61-81, wherein the engineered cell is CD45RA⁺ and CCR7⁺.~~

~~22. 83.~~—An *ex vivo* method of obtaining an engineered cell or population thereof, comprising:

- a. obtaining a cell; and
- b. genetically modifying the cell by inserting at least one sequence encoding a transgene within a safe harbor locus, wherein the safe harbor locus is ~~selected from~~ any one or more of ~~an~~ the sgRNA target loci selected from: chr11:128340000-128350000, chr10:33130000-33140000, chr10:72290000-72300000, chr11:65425000-65427000 (NEAT1), chr15:92830000-92840000, chr16:11220000-11230000, chr2:87460000-87470000, chr3:186510000-186520000, chr3:59450000-59460000, chr8:127980000-128000000, chr9:7970000-7980000, APRT, B2M, CAPNS1, CBLB, CD2, CD3E, CD3G, CD5, EDF1, FTL, PTEN, PTPN2, PTPN6, PTPRC, PTPRCAP, RPS23, RTRAF, SERF2, SLC38A1, SMAD2, SOCS1, SRP14, SRSF9, SUB1, TET2, TIGIT, TRAC, and TRIM28. ~~in Table 4.~~

~~23. 84.~~—The method of claim ~~83~~22, wherein obtaining the cell comprises: (i) collecting a tissue sample from a subject, (ii) isolating the cells from the tissue samples, and (iii) culturing the cells in vitro.

~~85. The method of claim 84, wherein the tissue sample is a blood sample.~~

~~24. 86.~~—The method of claim 22, ~~any one of claims 83-85~~, wherein the cell is a stem cell, a natural killer (NK) cell, an induced pluripotent stem cells (iPSC), a human pluripotent stem cell (HSPC), ~~a primary cell, an hematopoietic cell, an adaptive immune cell, an innate immune cell, a T cell, or a T cell progenitor.~~

~~87. The method of claim 86, wherein the cell is a T cell or a T cell progenitor.~~

~~88. The method of any one of claims 83-87, wherein the engineered cell is undifferentiated.~~

~~89. The method of any one of claims 83-88, wherein the engineered cell is CD45RA⁺ and CCR7⁺.~~

~~25. 90.~~—The method of claim 22, ~~any one of claims 83-89~~, wherein the at least one sequence is inserted using a homology-directed repair or a homology independent targeted insertion.

~~91.—The method of any one of claims 83-89, wherein the at least one sequence is inserted using a homology independent targeted insertion.~~

~~26.~~ **92.**—The method of claim 22~~any one of claims 83-91~~, wherein the genetically modifying in step (b) comprises contacting the cell with one or more guide ribonucleic acids (gRNAs), the at least one sequence, and one or more Cas9 endonucleases, wherein the one or more gRNAs and Cas9 endonucleases facilitate the insertion of the at least one sequence into chromosomal DNA within the safe harbor locus and wherein the one or more gRNAs comprises any one of SEQ ID NOS: 94, 102, 1-93, 95-101, or 103-120.

~~93.—The method of claim 92, wherein the one or more gRNAs comprises a sequence selected from any one of the sgRNA sequences in Table 4.~~

~~94.—The method of any one of claims 83-93, wherein the transgene encodes a recombinant protein, optionally a therapeutic agent.~~

~~95.—The method of any one of claims 83-93, wherein the transgene encodes a chimeric antigen receptor (CAR).~~

~~27.~~ **96.**—The method of claim ~~22~~**95**, wherein the at least one sequence comprises an exogenous promoter and the exogenous promoter is operably linked to the transgene.

~~97.—The method of claim 96, wherein the exogenous promoter is an EF1a promoter.~~

~~28.~~ **98.**—Use of the engineered cell of claim 1 in the manufacture of medicament for treating a disease, wherein the medicament is to be administered in an effective amount to A~~method of treating a subject having or at risk of having a~~the disease,~~comprising administering to the subject an effective amount of the cell of any one of claims 1-22, a population thereof, or the composition of claim 23.~~

~~99.—The method of claim 98, wherein the cell, the population thereof, or the composition is administered to the subject by infusion.~~

~~29.~~ **100.**—Use of a composition comprising the cell or population thereof in claim 22 in the manufacture of medicament for treating a disease, wherein the medicament is obtained using method of claim 22, wherein the medicament is to be administered in an effective amount A~~method of treating to~~a subject having or at risk of having a~~the disease,~~comprising:
a. ~~conducting the method of any one of claims 83-97; and~~
b. ~~administering to the subject an effective amount of a composition comprising the cell or a population thereof.~~

~~101.~~—The method of claim 100, wherein the composition is administered to the subject by infusion.

~~30.~~ ~~102.~~—The ~~method-use~~ of claim ~~100 or 101~~29, wherein the disease is cancer.

~~103.~~—The method of any one of claims 100-102 or , wherein the disease is blood cancer.

~~104.~~—A method of identifying a safe harbor locus, comprising:

- ~~a.~~—identifying genes or non-coding regions in a chromosome that are above a threshold level for expression across developmental cell states and/or a threshold level for accessibility of chromatin;
- ~~b.~~—generating a linear model that correlates the gene or non-coding region from step (a) with knock-in (KI) efficiency and estimates the KI efficiency of any gene or coding region on the chromosome; and
- ~~c.~~—selecting the safe harbor locus based on threshold parameters;
wherein the safe harbor locus is selected for insertion of at least one sequence encoding a transgene within a cell.

~~105.~~—The method of claim 104, wherein the threshold parameters include one or more of: stable expression of a transgene, knockout of the gene confers benefit to the function of the cell, no known function within the cell, stable transgene expression *in vitro* with or without CD3/CD28 stimulation, negligible off-target cleavage as detected by iGuide-Seq or CRISPR-Seq, less off-target cleavage relative to other loci as detected by iGuide-Seq or CRISPR-Seq, negligible transgene-independent cytotoxicity, negligible transgene-independent cytokine expression, negligible transgene-independent chimeric antigen receptor expression, negligible deregulation or silencing of nearby genes, and positioned outside of a cancer-related gene.

~~106.~~—The method of claim 105, wherein the stable expression of a transgene at the safe harbor locus is less than or equal to 2-fold expression change over the course of at least 1, 2, 3, 4, 5, 6, or 7 days, and wherein expression change is measured by mean fluorescence intensity of a reporter gene encoded by the at least one sequence.

~~107.~~—The method of any one of claims 104-106, wherein the accessibility of chromatin is measured using an assay for transposase-accessible chromatin using sequencing (ATAC-seq).

~~108.~~—The method of any one of claims 104-107, wherein the level of expression across developmental cell states is measured using RNA sequencing (RNA-seq).

~~109.—The method of any one of claims 104–108, wherein the cell is a stem cell, a human cell, a primary cell, an hematopoietic cell, an adaptive immune cell, an innate immune cell, a T cell or T cell progenitor.~~

~~110.—The method of any one of claims 104–109, wherein the linear model has a coefficient of determination (R^2 value) of at least 30%.~~

~~111.—The engineered cell, composition, gRNA or method of any one of the preceding claims, wherein insertion within the safe harbor locus increase cell cytotoxicity of diseased cells.~~

~~112.—The engineered cell, composition, gRNA or method of any one of the preceding claims wherein knock-in efficiency at the safe harbor locus is increased relative to other locations along the chromosome.~~

~~113.—An engineered cell, comprising at least one sequence encoding a transgene, wherein the at least one sequence is inserted within a safe harbor locus, wherein the safe harbor locus is at any one or more of an sgRNA target loci; and wherein expression of the at least one sequence encoding the transgene is operatively linked to an endogenous promoter or an exogenous promoter, and wherein the engineered cell is undifferentiated.~~

~~114.—The engineered cell of claim 113, wherein the safe harbor locus is selected any one of the integration sites designated: GS94, GS88, GS89, GS90, GS91, GS92, GS93, GS95, GS96, GS97, GS98, GS99, GS100, GS101, GS102, GS103, GS104, GS105, GS106, GS107, GS108, GS109, GS110, GS111, GS112, GS113, GS114, GS115, GS116, GS117, GS118, GS119, or GS120.~~

~~115.—The engineered cell of claim 113 or 114, wherein the safe harbor locus is the GS94 integration site.~~

~~116.—The engineered cell of claim 113, wherein the sgRNA target locus is selected from: chr11:128340000–128350000, chr10:33130000–33140000, chr10:72290000–72300000, chr11:65425000–65427000 (NEAT1), chr15:92830000–92840000, chr16:11220000–11230000, chr2:87460000–87470000, chr3:186510000–186520000, chr3:59450000–59460000, chr8:127980000–128000000, or chr9:7970000–7980000.~~

~~117.—The engineered cell of claim 113, wherein the sgRNA target locus is a gene selected from: APRT, B2M, CAPNS1, CBLB, CD2, CD3E, CD3G, CD5, EDF1, FTL, PTEN, PTPN2, PTPN6, PTPRC, PTPRCAP, RPS23, RTRAF, SERF2, SLC38A1, SMAD2, SOCS1, SRP14, SRSF9, SUB1, TET2, TIGIT, TRAC, or TRIM28.~~

~~118.—The engineered cell of any one of claims 113–117, wherein the one or more gRNAs comprises any one of SEQ ID NOS: 1–120.~~

~~119.—The engineered cell of any one of claims 113–118, wherein the engineered cell is a stem cell, a human cell, a primary cell, an hematopoietic cell, an adaptive immune cell, an innate immune cell, a T cell or a T cell progenitor.~~

~~120.—The engineered cell of claim 119, wherein the cell is a T cell or a T cell T cell progenitor.~~

~~121.—The engineered cell of any one of claims 113–120, wherein the engineered cell is CD45RA⁺ and CCR7⁺.~~

~~122.—The engineered cell of any one of claims 113–121, wherein the transgene encodes a recombinant protein, optionally a therapeutic agent.~~

~~123.—The engineered cell of any one of claims 113–122, wherein the transgene encodes or a chimeric antigen receptor (CAR).~~

~~124.—A composition comprising the engineered cell of any one of claims 113–123 and a pharmaceutical excipient.~~

~~125.—A method of editing a cell having chromosomal DNA, comprising inserting at least one sequence encoding a transgene within a safe harbor locus in the chromosomal DNA of the cell, wherein the safe harbor locus is at any one or more of an sgRNA target loci; and wherein the engineered cell is undifferentiated.~~

~~126.—The method of claim 125, wherein the engineered cell is a stem cell, a human cell, a primary cell, an hematopoietic cell, an adaptive immune cell, an innate immune cell, a T cell or a T cell progenitor.~~

~~127.—The method of claim 125 or 126, wherein the cell is a T cell or a T cell progenitor.~~

~~128.—A method of editing a T cell, comprising contacting a T cell with one or more guide ribonucleic acids (gRNAs), at least one sequence encoding a transgene, and one or more Cas9 endonucleases, wherein the one or more gRNAs and Cas9 endonucleases facilitate the insertion of the at least one sequence into chromosomal DNA within a safe harbor locus.~~

~~129.—The method of any one of claims 125–128, wherein the safe harbor locus is selected from any one of the integration sites designated: GS88, GS89, GS90, GS91, GS92, GS93, GS94, GS95, GS96, GS97, GS98, GS99, GS100, GS101, GS102, GS103, GS104, GS105, GS106, GS107, GS108, GS109, GS110, GS111, GS112, GS113, GS114, GS115, GS116, GS117, GS118, GS119, or GS120.~~

~~130.—The method of any one of claims 125–128, wherein the safe harbor locus is the GS94 integration site.~~

~~131.—The method of any one of claims 125–128, wherein the sgRNA target locus is selected from: chr11:128340000–128350000, chr10:33130000–33140000, chr10:72290000–72300000, chr11:65425000–65427000 (NEAT1), chr15:92830000–92840000, chr16:11220000–11230000, chr2:87460000–87470000, chr3:186510000–186520000, chr3:59450000–59460000, chr8:127980000–128000000, or chr9:7970000–7980000.~~

~~132.—The method of any one of claims 125–128, wherein the sgRNA target locus is a gene selected from: *APRT*, *B2M*, *CAPNS1*, *CBLB*, *CD2*, *CD3E*, *CD3G*, *CD5*, *EDF1*, *FTL*, *PTEN*, *PTPN2*, *PTPN6*, *PTPRC*, *PTPRCAP*, *RPS23*, *RTRAF*, *SERF2*, *SLC38A1*, *SMAD2*, *SOCS1*, *SRP14*, *SRSF9*, *SUB1*, *TET2*, *TIGIT*, *TRAC*, or *TRIM28*.~~

~~133.—The method of any one of claims 125–132, wherein the one or more gRNAs comprises any one of SEQ ID NOS: 1–120.~~

~~134.—The method of any one of claims 125–133, wherein the engineered cell is CD45RA⁺ and CCR7⁺ after insertion of the at least one sequence into the safe harbor locus.~~

~~135.—The method of any one of claims 125–134, wherein the transgene encodes a recombinant protein, optionally a therapeutic agent.~~

~~136.—The method of any one of claims 125–135, wherein the transgene encodes a chimeric antigen receptor (CAR).~~

~~137.—An *ex vivo* method of obtaining an undifferentiated engineered cell or population thereof, comprising:~~

~~c.—obtaining a cell;~~

~~d.—genetically modifying the cell by inserting at least one sequence encoding a transgene within a safe harbor locus, wherein the engineered cell is undifferentiated.~~

~~138.—The method of claim 137 wherein obtaining the cell comprises: (i) collecting a tissue sample from a subject, (ii) isolating the cells from the tissue samples, and (iii) culturing the cells *in vitro*.~~

~~139.—The method of claim 138, wherein the tissue sample is a blood sample.~~

~~140.—The method of any one of claims 137–139 wherein the cell is a stem cell, a human cell, a primary cell, an hematopoietic cell, an adaptive immune cell, an innate immune cell, a T cell or T cell progenitor.~~

~~141.—The method of claim 140, wherein the cell is a T cell or a T cell progenitor.~~

~~142.—The method of any one of claims 137–142, wherein the engineered cell is CD45RA⁺ and CCR7⁺.~~

~~143.—The method of any one of claims 137–143, wherein the transgene encodes a recombinant protein, optionally a therapeutic agent.~~

~~144.—The method of any one of claims 137–143, wherein the transgene encodes a chimeric antigen receptor (CAR).~~

~~145.—A method of treating a subject having or at risk of having a disease, comprising administering to the subject an effective amount of the cell of any one of claims 113–123, a population thereof, or the composition of claim 124.~~

~~146.—A method of treating a subject having or at risk of having a disease, comprising:~~

~~e.—conducting the method of any one of claims 125–144; and~~

~~d.—administering to the subject an effective amount of a composition comprising the cell or a population thereof.~~

~~147.—The method of claim 146, wherein the composition is administered to the subject by infusion.~~

~~148.—The method of claim 146 or 147, wherein the disease is cancer.~~

31. An engineered cell of claim 1 for use in therapy.