

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 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*.
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. The engineered cell of claim 1, wherein expression of the at least one sequence encoding the transgene is operatively linked to an exogenous promoter.
4. The engineered cell of claim 1, wherein the 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 chr9:7970000-7980000.
5. The engineered cell of claim 1, wherein the target locus is chr11:128340000-128350000 or chr15:92830000-92840000.
6. The engineered cell of claim 1, wherein the 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*, and *TRIM28*.
7. The engineered cell of claim 1, wherein the safe harbor locus is the GS94 or GS102 integration site in Table 4.
8. The engineered cell of claim 3, wherein the exogenous promoter is an EF1a promoter.
9. The engineered cell of claim 1, wherein the engineered cell is a natural killer (NK) cell, an induced pluripotent stem cells (iPSC), a human pluripotent stem cell (HSPC), a T cell or a T cell progenitor.

10. The engineered cell of claim 1, wherein the transgene encodes a recombinant protein, a therapeutic agent, or a chimeric antigen receptor (CAR).
11. A composition comprising the engineered cell of claim 1 and a pharmaceutical excipient.
12. A guide ribonucleic acids (gRNA) for editing a cell at a safe harbor locus, wherein the gRNA comprises any one of SEQ ID NOS: 94, 102, 1-93, 95-101, or 103-120.
13. 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 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. The method of claim 13, wherein the 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 chr9:7970000-7980000.
15. The method of claim 13, wherein the target locus is chr11:128340000-128350000 or chr15:92830000-92840000.
16. The method of claim 13, wherein the 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*, and *TRIM28*.
17. The method of claim 13, wherein the transgene encodes a recombinant protein, a therapeutic agent, or a chimeric antigen receptor (CAR).
18. The method of claim 13, wherein the at least one sequence comprises an exogenous promoter and the exogenous promoter is operably linked to the transgene.
19. The method of claim 13, wherein the cell is a T cell or T cell progenitor.

- 20.** The method of claim 13, wherein the at least one sequence is inserted using a homology-directed repair or a homology independent targeted insertion.
- 21.** The method of claim 13, 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.
- 22.** An *ex vivo* method of obtaining an engineered cell or population thereof, comprising:
- obtaining a cell; and
 - genetically modifying the cell by inserting at least one sequence encoding a transgene within a safe harbor locus, wherein the safe harbor locus is at any one or more of 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*.
- 23.** The method of claim 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.
- 24.** The method of claim 22, 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 T cell or a T cell progenitor.
- 25.** The method of claim 22, wherein the at least one sequence is inserted using a homology-directed repair or a homology independent targeted insertion.
- 26.** The method of claim 22, 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.

- 27.** The method of claim 22, wherein the at least one sequence comprises an exogenous promoter and the exogenous promoter is operably linked to the transgene.
- 28.** 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 subject having or at risk of having the disease.
- 29.** 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 to a subject having or at risk of having the disease.
- 30.** The use of claim 29, wherein the disease is cancer.
- 31.** An engineered cell of claim 1 for use in therapy.