

CLAIMS

1. A pluripotent human stem cell, wherein the stem cell comprises:
 - (i) a genomic edit that results in loss of function of Cytokine Inducible SH2 Containing Protein (CISH) and
 - (ii) a genomic edit that results in a loss of function of ~~an agonist of the~~ TGF beta receptor II (TGF β RII), ~~signaling pathway~~, or a genomic edit that results in a loss of function of adenosine A2a receptor (ADORA2A).
2. The pluripotent human stem cell of claim 1, wherein the stem cell comprises a genomic edit that results in a loss of function of ~~an agonist of the~~ TGF beta receptor II (TGF β RII)~~signaling pathway~~ and a genomic edit that results in a loss of function of ADORA2A; and/or,
- ~~3. The pluripotent human stem cell of claim 1 or 2, wherein the stem cell comprises a genomic edit that results in a loss of function of a TGF beta receptor or a dominant negative variant of a TGF beta receptor.~~
- ~~4. The pluripotent human stem cell of claim 3, wherein the TGF beta receptor is a TGF beta receptor II (TGF β RII).~~
5. ~~The pluripotent human stem cell of any one of the preceding claims,~~ wherein the stem cell expresses one or more pluripotency markers selected from the group consisting of SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-1, Oct4, Rex1, and Nanog.
- ~~6. A differentiated cell, wherein the differentiated cell is a daughter cell of the pluripotent human stem cell of any one of the preceding claims.~~
- ~~7. The differentiated cell of claim 6, wherein the differentiated cell is an immune cell.~~

~~8. — The differentiated cell of claim 6, wherein the differentiated cell is a lymphocyte.~~

~~9. — The differentiated daughter cell of claim 6, wherein the differentiated cell is a natural killer cell.~~

~~10. — The differentiated cell of claim 6, wherein the stem cell is a human induced-pluripotent stem cell (iPSC), and wherein the differentiated daughter cell is an iNK cell.~~

~~11. — The differentiated cell of claim 6, wherein the cell:~~

~~(a) does not express endogenous CD3, CD4, and/or CDS; and~~

~~(b) expresses at least one endogenous gene encoding:~~

~~(i) CD56 (NCAM), CD49, CD43, and/or CD45, or any combination thereof;~~

~~(ii) NK cell receptor immunoglobulin gamma Fc region receptor III (FcyRIII, cluster of differentiation 16 (CD16));~~

~~(iii) natural killer group 2 member D (NKG2D);~~

~~(iv) CD69;~~

~~(v) a natural cytotoxicity receptor;~~

~~or any combination of two or more thereof.~~

~~12. — The cell of any of the preceding claims, wherein the cell comprises one or more additional genomic edits.~~

~~13. — The cell of claim 12, wherein the cell:~~

~~(1) comprises at least one genomic edit characterized by an exogenous nucleic acid expression construct that comprises a nucleic acid sequence encoding:~~

~~(i) a chimeric antigen receptor (CAR);~~

~~(ii) a FcyRIII (CD16) or a variant of FcyRIII (CD16);~~

~~(iii) interleukin 15 (IL-15);~~

~~(iv) an IL-15 receptor (IL-15R) agonist, or a constitutively active variant of an IL-15 receptor;~~

~~(v) an IL-12 receptor (IL-12R) agonist, or a constitutively active variant of an IL-12 receptor;~~

~~(vi) an IL-12 receptor (IL-12R) agonist, or a constitutively active variant of an IL-12 receptor;~~

~~(vii) human leukocyte antigen G (HLA-G);~~

~~(viii) human leukocyte antigen E (HLA-E);~~

~~(ix) leukocyte surface antigen cluster of differentiation CD47 (CD47);-
or any combination of two or more thereof;~~

and/or

~~(2) comprises at least one genomic edit that results in a loss of function of at least one of:~~

~~(i) ADORA2A;~~

~~(ii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);~~

~~(iii) 2 microglobulin (B2M);~~

~~(iv) programmed cell death protein 1 (PD-1);~~

~~(v) class II, major histocompatibility complex, transactivator (CIITA);~~

~~(vi) natural killer cell receptor NKG2A (natural killer group 2A);~~

~~(vii) two or more HLA class II histocompatibility antigen alpha chain genes,
and/or two or more HLA class II histocompatibility antigen beta chain genes;~~

~~(viii) cluster of differentiation 32B (CD32B, FCGR2B);~~

~~(ix) T cell receptor alpha constant (TRAC);-~~

~~or any combination of two or more thereof.~~

14.3. A human induced pluripotent stem cell (iPSC), wherein the iPSC comprises a genomic edit that results in a loss of function of adenosine A2a receptor (ADORA2A).

15.4. The human iPSC of claim 314, wherein the iPSC comprises a genomic edit that results in a loss of function of ~~an agonist of the~~ TGF beta receptor II (TGFβRII)~~signaling-pathway~~ or a genomic edit that results in loss of function of Cytokine Inducible SH2 Containing Protein (CISH), and optionally:-

~~16. The human iPSC of claim 15,~~ wherein the iPSC comprises a genomic edit that results in a loss of function of ~~an agonist of the~~ TGF beta receptor II (TGFβRII) signaling pathway and a genomic edit that results in loss of function of CISH; and/or

~~17. The human iPSC of claim 15 or 16, wherein the iPSC comprises a genomic edit that results in a loss of function of a TGF beta receptor or a dominant negative variant of a TGF beta receptor.~~

~~18. The human iPSC of claim 17, wherein the TGF beta receptor is a TGF beta receptor II (TGFβRII).~~

~~19. The human iPSC of any one of claims 14-18,~~ wherein the iPSC expresses one or more pluripotency markers selected from the group consisting of SSEA-3, SSEA-4, TRA- 1-60, TRA-1-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-1, Oct4, Rex1, and Nanog.

5. A pluripotent human stem cell, wherein the stem cell comprises a genomic edit that results in a loss of function of TGF beta receptor II (TGFβRII); and optionally further comprising a loss of function of an antagonist of interleukin signaling; and/or wherein the stem cell further comprises a genomic modification that results in the loss of function of an antagonist of interleukin signaling; and/or wherein the antagonist of interleukin signaling is an antagonist of the IL-15 signaling pathway and/or of the IL-2 signaling pathway; and/or comprising a loss of function of Cytokine Inducible SH2 Containing Protein (CISH); and optionally wherein the stem cell comprises a genomic modification that results in the loss of function of CISH; and/or wherein the stem cell expresses one or more pluripotency markers selected from the group consisting of SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-1, Oct4, Rex1, and Nanog.

20.6. A differentiated cell,

(i) wherein the differentiated cell is a daughter cell of the pluripotent human stem cell of any one of claims 1-2, or

(ii) wherein the differentiated cell is a daughter cell of the human iPSC of any one of claims 314-419, or

(iii) wherein the differentiated cell is a daughter cell of the pluripotent human stem cell of claim 5.

21.7. The differentiated cell of claim 620, wherein the differentiated cell is an immune cell;

~~22. The differentiated cell of claim 20, wherein or wherein the differentiated cell is a lymphocyte.~~

~~23. The differentiated daughter cell of claim 20, or wherein the differentiated cell is a natural killer cell.~~

~~24. The differentiated cell of claim 20, or wherein the stem cell is a human induced pluripotent stem cell (iPSC), and wherein the differentiated daughter cell is an iNK cell.~~

~~25. The differentiated cell of claim 20, wherein the cell:~~
~~(a) does not express endogenous CD3, CD4, and/or CDS; and~~

~~(b) expresses at least one endogenous gene encoding:~~

~~(i) CD56 (NCAM), CD49, CD43, and/or CD45, or any combination thereof;~~

~~(ii) NK cell receptor immunoglobulin gamma Fc region receptor III (FcγRIII, cluster of differentiation 16 (CD16));~~

~~(iii) natural killer group 2 member D (NKG2D);~~

~~(iv) CD69;~~

~~(v) a natural cytotoxicity receptor;~~

~~or any combination of two or more thereof.~~

26.8. The cell of any ~~of one of the of claims 14-25~~preceding claims, wherein the cell comprises one or more additional genomic edits.

~~27. The cell of claim 26, wherein the cell:~~

~~(1) comprises at least one genomic edit characterized by an exogenous nucleic acid expression construct that comprises a nucleic acid sequence encoding:~~

~~(i) a chimeric antigen receptor (CAR);~~

~~(ii) a FcγRIII (CD16) or a variant of FcγRIII (CD16);~~

~~(iii) interleukin 15 (IL-15);~~

~~(iv) an IL-15 receptor (IL-15R) agonist, or a constitutively active variant of an IL-15 receptor;~~

~~(v) an IL-12 receptor (IL-12R) agonist, or a constitutively active variant of an IL-12 receptor;~~

~~(vi) an IL-12 receptor (IL-12R) agonist, or a constitutively active variant of an IL-12 receptor;~~

~~(vii) human leukocyte antigen G (HLA-G);~~

~~(viii) human leukocyte antigen E (HLA-E);~~

~~(ix) leukocyte surface antigen cluster of differentiation CD47 (CD47);~~

~~or any combination of two or more thereof;~~

~~and/or~~

~~(2) comprises at least one genomic edit that results in a loss of function of at least one~~

~~of:~~

~~(i) cytokine inducible SH2-containing protein (CISH);~~

~~(ii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);~~
~~(iii) 2 microglobulin (B2M);~~
~~(iv) programmed cell death protein 1 (PD-1);~~
~~(v) class II, major histocompatibility complex, transactivator (CIITA);~~
~~(vi) natural killer cell receptor NKG2A (natural killer group 2A);~~
~~(vii) two or more HLA class II histocompatibility antigen alpha chain genes,~~
~~and/or two or more HLA class II histocompatibility antigen beta chain genes;~~
~~(viii) cluster of differentiation 32B (CD32B, FCGR2B);~~
~~(ix) T cell receptor alpha constant (TRAC);~~
~~or any combination of two or more thereof.~~

28.9. The cell of any one of claims 1-~~427~~, claim 6 parts (i), and (ii), and claims 7-8, wherein:

the genomic edit resulting in loss of function of CISH was produced using a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 258-364, 1155, and 1162;

the genomic edit resulting in loss of function of TGF β RII was produced using a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 29-257, 1157, and 1161; and/or

the genomic edit resulting in loss of function of ADORA2A was produced using a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 827-1143, 1159, and 1163-; and/or wherein:

~~29. — The cell of any one of claims 1-28, wherein:~~

the genomic edit resulting in loss of function of CISH was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease and (ii) a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 258-364, 1155, and 1162;

the genomic edit resulting in loss of function of TGF β RII was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease and (ii) a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 29-257, 1157, and 1161; and/or

the genomic edit resulting in loss of function of ADORA2A was produced using a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease and (ii) a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 827-1143, 1159, and 1163.

30.10. A method of making the cell of any one of claims 1-~~429~~, and claim 6, parts(i) and (ii) and claims 7-9, the method comprising contacting the cell with one or more of:

(i) an RNA-guided nuclease and a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 258-364, 1155, and 1162;

an RNA-guided nuclease and a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 29-257, 1157, and 1161; and/or

an RNA-guided nuclease and a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 827-1143, 1159, and 1163-; or

~~31. — A method of making the cell of any one of claims 1-30, the method comprising contacting the cell with one or more of:~~

(ii) a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease and (ii) a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 258-364, 1155, and 1162;

a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease and (ii) a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 29-257, 1157, and 1161; and/or

a ribonucleoprotein (RNP) complex comprising (i) an RNA-guided nuclease and (ii) a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence according to any one of SEQ ID NO: 827-1143, 1159, and 1163.

32.11. The cell of claim 9 or the method of ~~any one of claims 1029-31~~, wherein the RNA-guided nuclease is a Cas12a variant- and optionally

~~33. The method of claim 32,~~ wherein the Casl2a variant comprises one or more amino acid substitutions selected from M537R, F870L, and H800A; or

~~34. The method of claim 32,~~ wherein the Casl2a variant comprises amino acid substitutions M537R, F870L, and H800A; or

~~35. The method of claim 32,~~ wherein the Casl2a variant comprises the amino acid sequence of SEQ ID NO:1148; and/or

~~36. The method of any one of claims 30-35,~~ comprising contacting the cell with:

(i) a guide RNA comprising a targeting domain sequence comprising the nucleotide sequence of SEQ ID NO: 1155 or 1162; a guide RNA comprises a targeting domain sequence comprising the nucleotide sequence of SEQ ID NO: 1157 or 1161; and a guide RNA comprises a targeting domain sequence comprising the nucleotide sequence of SEQ ID NO: 1159 or 1163; and

(ii) an RNA-guided nuclease comprising the amino acid sequence of one of SEQ ID NO:1144-1151 (or a portion thereof).

~~37. A pluripotent human stem cell, wherein the stem cell comprises a disruption in the transforming growth factor beta (TGF beta) signaling pathway.~~

~~38. The pluripotent human stem cell of claim 34, wherein the stem cell comprises a genomic edit that results in a loss of function of an agonist of the TGF beta signaling pathway.~~

~~39. The pluripotent human stem cell of claim 37 or 38, comprising a loss of function of a TGF beta receptor or a dominant negative variant of a TGF beta receptor.~~

~~40. The pluripotent human stem cell of claim 39, wherein the TGF beta receptor is a TGF beta receptor II (TGFβRII).~~

~~41. — The pluripotent human stem cell of any one of claims 37-40, further comprising a loss of function of an antagonist of interleukin signaling.~~

~~42. — The pluripotent human stem cell of any one of claims 37-41, wherein the stem cell further comprises a genomic modification that results in the loss of function of an antagonist of interleukin signaling.~~

~~43. — The pluripotent human stem cell of claim 41 or 42, wherein the antagonist of interleukin signaling is an antagonist of the IL-15 signaling pathway and/or of the IL-2 signaling pathway.~~

~~44. — The pluripotent human stem cell of any one of claims 37-43, comprising a loss of function of Cytokine Inducible SH2 Containing Protein (CISH).~~

~~45. — The pluripotent human stem cell of claim 44, wherein the stem cell comprises a genomic modification that results in the loss of function of **CISH**.~~

~~46. — The pluripotent human stem cell of any one of claims 37-45, wherein the stem cell expresses one or more pluripotency markers selected from the group consisting of SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-1, Oct4, Rex1, and Nanog.~~

~~47. — A differentiated cell, wherein the differentiated cell is a daughter cell of the pluripotent human stem cell of any one of claims 37-46.~~

~~48. — The differentiated cell of claim 47, wherein the differentiated cell is an immune cell.~~

~~49. — The differentiated cell of claim 47, wherein the differentiated cell is a lymphocyte.~~

50. — ~~The differentiated daughter cell of claim 47, wherein the differentiated cell is a natural killer cell.~~

51. — ~~The differentiated cell of claim 47, wherein the stem cell is a human induced pluripotent stem cell (iPSC), and wherein the differentiated daughter cell is an iNK cell.~~

52. — ~~The differentiated cell of claim 47, wherein the cell:~~

~~(a) does not express endogenous CD3, CD4, and/or CDS; and~~

~~(b) expresses at least one endogenous gene encoding:~~

~~(i) CD56 (NCAM), CD49, CD43, and/or CD45, or any combination thereof;~~

~~(ii) NK cell receptor immunoglobulin gamma Fc region receptor III (FcyRIII, cluster of differentiation 16 (CD16));~~

~~(iii) natural killer group 2 member D (NKG2D);~~

~~(iv) CD69;~~

~~(v) a natural cytotoxicity receptor;~~

~~or any combination of two or more thereof.~~

53. — ~~The cell of any of claims 37-52, wherein the cell comprises one or more additional genomic edits.~~

54. — ~~The cell of claim 53, wherein the cell:~~

~~(1) comprises at least one genomic edit characterized by an exogenous nucleic acid expression construct that comprises a nucleic acid sequence encoding:~~

~~(i) a chimeric antigen receptor (CAR);~~

~~(ii) a FcyRIII (CD16) or a variant of FcyRIII (CD16);~~

~~(iii) interleukin 15 (IL-15);~~

~~(iv) an IL-15 receptor (IL-15R) agonist, or a constitutively active variant of an IL-15 receptor;~~

~~(v) an IL-12 receptor (IL-12R) agonist, or a constitutively active variant of an IL-12 receptor;~~

~~(vi) an IL-12 receptor (IL-12R) agonist, or a constitutively active variant of an IL-12 receptor;~~

- ~~(vii) human leukocyte antigen G (HLA-G);~~
- ~~(viii) human leukocyte antigen E (HLA-E);~~
- ~~(ix) leukocyte surface antigen cluster of differentiation CD47 (CD47);-~~
- ~~or any combination of two or more thereof;~~

~~and/or~~

~~(2) comprises at least one genomic edit that results in a loss of function of at least one of;~~

- ~~(i) cytokine inducible SH2-containing protein (CISH);~~
- ~~(ii) adenosine A2a receptor (ADORA2A);~~
- ~~(iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);~~
- ~~(iv) 2 microglobulin (B2M);~~
- ~~(v) programmed cell death protein 1 (PD-1);~~
- ~~(vi) class II, major histocompatibility complex, transactivator (CIITA);~~
- ~~(vii) natural killer cell receptor NKG2A (natural killer group 2A);~~
- ~~(viii) two or more HLA class II histocompatibility antigen alpha chain genes,~~
- ~~and/or two or more HLA class II histocompatibility antigen beta chain genes;~~
- ~~(ix) cluster of differentiation 32B (CD32B, FCGR2B);~~
- ~~(x) T cell receptor alpha constant (TRAC);-~~
- ~~or any combination of two or more thereof.~~

55.12. A method of culturing a pluripotent human stem cell, comprising culturing the stem cell in a medium comprising activin.

56.13. The method of claim 1255, wherein the pluripotent human stem cell is an embryonic stem cell or an induced pluripotent stem cell; and/or:-

~~57.——The method of claim 55 or 56, wherein the pluripotent human stem cell does not express TGFβRII.~~

~~58.——The method of any one of claims 55-57, wherein the pluripotent human stem cell is genetically engineered not to express TGFβRII.~~

~~59. The method of any one of claims 55-57,~~ wherein the pluripotent human stem cell is genetically engineered to knock out a gene encoding TGF β RII; and/or

~~60. The method of any one of claims 55-59,~~ wherein the activin is activin A; and/or

~~61. The method of any one of claims 55-60,~~ wherein the medium does not comprise TGF β ; and/or

~~62. The method of any one of claims 55-61,~~ wherein the culturing is performed for a defined period of time (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 days, or more); and/or-

~~63. The method of any one of claims 55-62,~~ wherein at one or more times during or following the culturing step, the pluripotent human stem cell maintains pluripotency (e.g., exhibits one or more pluripotency markers); and optionally

~~64. The method of claim 63,~~ wherein at one or more times during or following the culturing step, the pluripotent human stem cell expresses a detectable level of one or more of SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-1, Oct4, Rex1, and Nanog; or

~~65. The method of claim 63,~~ wherein at a time during or following the culturing step, the pluripotent human stem cell is differentiated into cells of endoderm, mesoderm, and/or ectoderm lineage; and optionally

~~66. The method of claim 65,~~ wherein the pluripotent human stem cell, or its progeny, is further differentiated into a natural killer (NK) cell.

~~67. The method of any one of claims 55-66,~~ wherein the pluripotent human stem cell;

~~(1) comprises at least one genomic edit characterized by an exogenous nucleic acid expression construct that comprises a nucleic acid sequence encoding:~~

~~(i) a chimeric antigen receptor (CAR);~~

- (ii) a FcγRIII (CD16) or a variant of FcγRIII (CD16);
- (iii) interleukin 15 (IL-15);
- (iv) an IL-15 receptor (IL-15R) agonist, or a constitutively active variant of an IL-15 receptor;
- (v) an IL-12 receptor (IL-12R) agonist, or a constitutively active variant of an IL-12 receptor;
- (vi) an IL-12 receptor (IL-12R) agonist, or a constitutively active variant of an IL-12 receptor;
- (vii) human leukocyte antigen G (HLA-G);
- (viii) human leukocyte antigen E (HLA-E);
- (ix) leukocyte surface antigen cluster of differentiation CD47 (CD47);
- or any combination of two or more thereof;

and/or

(2) comprises at least one genomic edit that results in a loss of function of at least one of:

- (i) cytokine inducible SH2-containing protein (CISH);
- (ii) adenosine A2a receptor (ADORA2A);
- (iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);
- (iv) 2 microglobulin (B2M);
- (v) programmed cell death protein 1 (PD-1);
- (vi) class II, major histocompatibility complex, transactivator (CIITA);
- (vii) natural killer cell receptor NKG2A (natural killer group 2A);
- (viii) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes;
- (ix) cluster of differentiation 32B (CD32B, FCGR2B);
- (x) T cell receptor alpha constant (TRAC);
- or any combination of two or more thereof.

68.14. A cell culture comprising (i) a pluripotent human stem cell and (ii) a cell culture medium comprising activin, wherein the pluripotent human stem cell comprises a genomic edit that results in disruption in the transforming growth factor beta-a loss of function of (TGF beta receptor II)-signaling pathway(TGFβRII).

~~69. The cell culture of claim 68, wherein the stem cell comprises a genomic edit that results in a loss of function of an agonist of the TGF beta signaling pathway.~~

~~70. The cell culture of claim 69, wherein the genomic edit is a genomic edit.~~

~~71. The cell culture of any one of claims 68-70, wherein the stem cell comprises a loss of function of a TGF beta receptor or a dominant negative variant of a TGF beta receptor.~~

~~72. The cell culture of claim 71, wherein the TGF beta receptor is a TGF beta receptor II (TGFβRII).~~

~~73. The cell culture of any one of claims 68-72, wherein the pluripotent human stem cell:~~

~~(1) comprises at least one genomic edit characterized by an exogenous nucleic acid expression construct that comprises a nucleic acid sequence encoding:~~

~~(i) a chimeric antigen receptor (CAR);~~

~~(ii) a FcγRIII (CD16) or a variant of FcγRIII (CD16);~~

~~(iii) interleukin 15 (IL-15);~~

~~(iv) an IL-15 receptor (IL-15R) agonist, or a constitutively active variant of an IL-15 receptor;~~

~~(v) an IL-12 receptor (IL-12R) agonist, or a constitutively active variant of an IL-12 receptor;~~

~~(vi) an IL-12 receptor (IL-12R) agonist, or a constitutively active variant of an IL-12 receptor;~~

~~(vii) human leukocyte antigen G (HLA-G);~~

~~(viii) human leukocyte antigen E (HLA-E);~~

~~(ix) leukocyte surface antigen cluster of differentiation CD47 (CD47);~~

~~or any combination of two or more thereof;~~

~~and/or~~

~~(2) comprises at least one genomic edit that results in a loss of function of at least one of:~~

(i) ~~cytokine inducible SH2-containing protein (CISH);~~
(ii) ~~adenosine A2a-receptor (ADORA2A);~~
(iii) ~~T cell immunoreceptor with Ig and ITIM domains (TIGIT);~~
(iv) ~~2 microglobulin (B2M);~~
(v) ~~programmed cell death protein 1 (PD-1);~~
(vi) ~~class II, major histocompatibility complex, transactivator (CIITA);~~
(vii) ~~natural killer cell receptor NKG2A (natural killer group 2A);~~
(viii) ~~two or more HLA class II histocompatibility antigen alpha chain genes,~~
~~and/or two or more HLA class II histocompatibility antigen beta chain genes;~~
(ix) ~~cluster of differentiation 32B (CD32B, FCGR2B);~~
(x) ~~T cell receptor alpha constant (TRAC);~~
~~or any combination of two or more thereof.~~

74.15. A method of increasing a level of iNK cell activity comprising:

(i) providing a pluripotent human stem cell comprising a
~~disruption genomic edit that results in a~~ loss of function of transforming growth-
factor beta (TGF beta receptor II (TGFβRII)) signaling pathway; and
(ii) differentiating the pluripotent human stem cell into an iNK cell,
wherein the iNK cell has a higher level of cell activity as compared to an iNK cell not
comprising a ~~disruption genomic edit that results in a loss of function of the~~ TGF beta signaling-
pathway receptor II (TGFβRII).

75.16. The method of claim 1574, wherein the iNK is differentiated from a
pluripotent human stem cell cultured in a medium comprising activin; and/or-

76. ~~The method of claim 74 or 75,~~ further comprising culturing the pluripotent
human stem cell in a medium comprising activin before and/or during the differentiating
step.

77. ~~The method of any one of claims 74-76, further comprising disrupting the~~
~~transforming growth factor beta (TGF beta) signaling pathway in the pluripotent human stem~~
~~cell.~~

~~78. The method of any one of claims 73-77, wherein the stem cell comprises a genomic edit that results in a loss of function of an agonist of the TGF beta signaling pathway.~~

~~79. The method of any one of claims 73-78, wherein the stem cell comprises a loss of function of a TGF beta receptor or a dominant negative variant of a TGF beta receptor.~~

~~80. The method of claim 79, wherein the TGF beta receptor is a TGF beta receptor-II (TGFβRII).~~

~~81. The method of any one of claims 73-80, wherein the pluripotent human stem cell:~~

- ~~(1) comprises at least one genomic edit characterized by an exogenous nucleic acid expression construct that comprises a nucleic acid sequence encoding:~~
- ~~(i) a chimeric antigen receptor (CAR);~~
 - ~~(ii) a FcγRIII (CD16) or a variant of FcγRIII (CD16);~~
 - ~~(iii) interleukin 15 (IL-15);~~
 - ~~(iv) an IL-15 receptor (IL-15R) agonist, or a constitutively active variant of an IL-15 receptor;~~
 - ~~(v) an IL-12 receptor (IL-12R) agonist, or a constitutively active variant of an IL-12 receptor;~~
 - ~~(vi) an IL-12 receptor (IL-12R) agonist, or a constitutively active variant of an IL-12 receptor;~~
 - ~~(vii) human leukocyte antigen-G (HLA-G);~~
 - ~~(viii) human leukocyte antigen-E (HLA-E);~~
 - ~~(ix) leukocyte surface antigen cluster of differentiation CD47 (CD47); or any combination of two or more thereof;~~

~~and/or~~

~~(2) comprises at least one genomic edit that results in a loss of function of at least one~~

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~~(i) cytokine inducible SH2-containing protein (CISH);~~

(ii) adenosine A2a-receptor (ADORA2A);
(iii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);
(iv) 2-microglobulin (B2M);
(v) programmed cell death protein 1 (PD-1);
(vi) class II, major histocompatibility complex, transactivator (CIITA);
(vii) natural killer cell receptor NKG2A (natural killer group 2A);
(viii) two or more HLA class II histocompatibility antigen alpha chain genes,
and/or two or more HLA class II histocompatibility antigen beta chain genes;
(ix) cluster of differentiation 32B (CD32B, FCGR2B);
(x) T cell receptor alpha constant (TRAC);
or any combination of two or more thereof.

82.17. A method of treating a subject having or at risk of cancer, the method comprising administering to the subject Use of the cell of any one of claims 76-1013, 20-29, or 47-54, in the manufacture of a medicament for the treatment of a subject having or at risk of cancer, wherein the medicament is to be administered to the subject, thereby treating the cancer in the subject.