CLAIMS

1. A method of generating genome engineered iPSCs comprising at least one targeted genomic editing, in which the genome-engineered iPSCs are capable of differentiating into an iPSC-derived hematopoietic lineage cell, the method comprising (I), (II) or (III):

(I): genetically engineering iPSCs comprising:

(i) knocking out a T cell receptor (TCR) by disrupting a polynucleic acid encoding a constant region of TCR alpha (TRAC); and

(ii) knocking in a polynucleotide encoding at least one chimeric antigen receptor (CAR) at the polynucleic acid encoding the constant region of the TCR alpha, the polynucleotide encoding the at least one CAR being expressed under control of an endogenous TCR promoter of the TCR alpha constant region;

thereby obtaining the genome-engineered iPSCs capable of differentiating into an iPSC-derived hematopoietic lineage cell;

(II): genetically engineering reprogramming non-pluripotent cells to obtain the genome-engineered iPSCs comprising:

(i) contacting T cells with one or more reprogramming factors, and optionally a small molecule composition comprising a TGFβ receptor/ALK inhibitor, a MEK inhibitor, a GSK3 inhibitor and/or a ROCK inhibitor to initiate reprogramming of the T cells; and

(ii) genetically engineering the reprogramming non-pluripotent cells of step (II)(i) comprising (a) and (b), in any order:

(a) knocking out a T cell receptor (TCR) by disrupting a polynucleic acid encoding a constant region of TCR alpha (TRAC);

(b) knocking in a polynucleotide encoding at least one chimeric antigen receptor (CAR) at the polynucleic acid encoding the constant region of the TCR alpha, the polynucleotide encoding the at least one CAR being expressed under control of an endogenous TCR promoter of the TCR alpha constant region;

in which reprogramming to iPSCs is completed after the genetically engineering, thereby obtaining the genome-engineered iPSCs capable of differentiating into an iPSC-derived hematopoietic lineage cell;

and,

(III): genetically engineering T cells for reprogramming to obtain genome-engineered iPSCs comprising (i) and (ii):

(i) genetically engineering the T cells comprising (a) and (b), in any order:

(a) knocking out a T cell receptor (TCR) by disrupting a polynucleic acid encoding a constant region of TCR alpha (TRAC);

(b) knocking in a polynucleotide encoding at least one chimeric antigen receptor (CAR) at the polynucleic acid encoding the constant region of the TCR alpha, the polynucleotide encoding the at least one CAR being expressed under control of an endogenous TCR promoter of the TCR alpha constant region;

(ii) contacting the cells of step (III)(i) with one or more reprogramming factors, and optionally a small molecule composition comprising a TGFβ receptor/ALK inhibitor, a MEK inhibitor, a GSK3 inhibitor and/or a ROCK inhibitor, to obtain genome-engineered iPSCs;

thereby obtaining the genome-engineered iPSCs capable of differentiating into an iPSC-derived hematopoietic lineage cell.

2. A method according to Claim 1, in which the genome-engineered iPSCs further comprise: (i) insertion of one or more exogenous polynucleotides; or (ii) in/del at one or more endogenous genes,

in which the one or more exogenous polynucleotides encode safety switch proteins, targeting modalities, receptors, signaling molecules, transcription factors, pharmaceutically active proteins and peptides, drug target candidates, immune response regulation and modulation, or proteins promoting or suppressing engraftment, trafficking, homing, viability, self-renewal, persistence, and/or survival of the iPSCs or derivative cells thereof.

3. A method according to Claim 2, in which:

(i) the exogenous polynucleotides are operatively linked to (1) one or more exogenous promoters comprising CMV, EF1α, PGK, CAG, UBC, or other constitutive, inducible, temporal-, tissue-, or cell type- specific promoters; or (2) one or more endogenous promoters comprised in a selected site comprising AAVS1, CCR5, ROSA26, collagen, HTRP, H11, beta-2 microglobulin, GAPDH, TCR or RUNX1;

(ii) the exogenous polynucleotides encode proteins comprising caspase, thymidine kinase, cytosine deaminase, modified EGFR, B-cell CD20, or any combinations thereof, in which the exogenous polynucleotides are integrated in different safe harbor locus comprising AAVS1, CCR5, ROSA26, collagen, HTRP, H11, beta-2 microglobulin, GAPDH, TCR or RUNX1;

(iii) the endogenous gene comprises at least one of B2M, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, CIITA, RFX5, RFXAP, and any gene in the chromosome 6p21 region; and/or

(iv) the genome-engineered iPSCs comprise a caspase encoding exogenous polynucleotide at AAVS1 locus, and a thymidine kinase encoding exogenous polynucleotide at H11 locus.

4. A method according to Claim 1, in which group (I), (II) and/or (III) further comprising: contacting the genome-engineered iPSCs with a small molecule composition comprising a MEK inhibitor, a GSK3 inhibitor and a ROCK inhibitor, and optionally comprising clonal expansion of the genome-engineered iPSCs.

5. A method according to Claim 2, in which the method has at least one of the following characteristics:

(i) in which the one or more exogenous polynucleotides are operatively linked to one or more exogenous promoters comprised in a construct, or to an endogenous promoter comprised in a selected site upon integration;

(ii) in which the one or more exogenous polynucleotides of interest are comprised in a construct that further comprises a pair of homology arms specific to a selected site and flanking the exogenous polynucleotides;

(iii) in which the one or more exogenous polynucleotides remain integrated and functional in subsequently expanded iPSCs, differentiated cells derived from the genome-engineered iPSCs, and/or dedifferentiated cells derived therefrom;

(iv) in which the one or more exogenous polynucleotides are linked to each other by a linker sequence; or in which the one or more exogenous polynucleotides expresses under same or different promoters;

(v) in which constructs comprising the one or more exogenous polynucleotides further comprise at least one polynucleotide encoding a marker;

(vi) in which constructs comprising the one or more exogenous polynucleotides further comprise a polynucleotide encoding a marker that is driven by an endogenous promoter at a selected site; and

(vii) in which the one or more exogenous polynucleotides of interest encodes safety switch protein and is operatively linked to CAG.

6. A method according to Claim 5, in which the method has at least one of the following characteristics:

(i) in which the linker sequence (a) encodes a self-cleaving peptide; or (b) provides an Internal Ribosome Entry Sequence (IRES);

(ii) in which the selected site is a safe harbor locus, highly expressive locus, temporally expressed locus, or a gene locus for interruption, in which at least one of the exogenous polynucleotides operatively linked to an exogenous promoter comprising CMV, EF1α, PGK, CAG, UBC, or other constitutive, inducible, temporal-, tissue-, or cell type- specific promoters;

(iii) in which the selected site comprises AAVS1, CCR5, ROSA26, collagen, HTRP, H11, beta-2 microglobulin, GAPDH, TCR or RUNX1, loci meeting the criteria of a genome safe harbor, B2M, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, CIITA, RFX5, RFXAP, and any gene in the chromosome 6p21 region;

7. A method of generating genome-engineered non-pluripotent cells, the method comprising:

(i) obtaining genome-engineered iPSCs according to any preceding claim; and

(ii) differentiating the genome-engineered iPSCs to obtain derived non-pluripotent cells comprising the same genomic editing comprised in the genome-engineered iPSCs.

8. A method according to Claim 7, in which the derived non-pluripotent cells comprise hematopoietic lineage cells; and in which the differentiating step (ii) is without formation of embryoid bodies.

9. A method according to Claim 7, in which the derived non-pluripotent cells comprise mesodermal cells, CD34 cells, hemogenic endothelium cells, hematopoietic stem and progenitor cells, hematopoietic multipotent progenitor cells, T cell progenitors, NK cell progenitors, T cells, NKT cells, NK cells, or B cells.

10. Genome-engineered iPSCs generated from a method according to any of Claims 1 to 6.

11. Genome-engineered iPSCs according to Claim 10, in which the iPSCs comprise at least one of the following:

(i) one or more in/dels comprised in an endogenous gene associated with targeting modalities; receptors; signaling molecules; transcription factors; drug target candidates; immune response regulation and modulation; and proteins suppressing engraftment, trafficking, homing, viability, self-renewal, persistence, and/or survival of the genome-engineered iPSCs or derivative cells thereof;

(ii) deletion or reduced expression in at least one of B2M, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, CIITA, RFX5, RFXAP and any gene in the chromosome 6p21 region;

(iii) introduced or increased expression in at least one of HLA-E, HLA-G, CD16, 41BBL, CD3, CD4, CD8, CD47, CD113, CD131, CD137, CD80, PDL1, $A_{2A}R$, CAR, TCR, Fc receptor, an engager, and a surface triggering receptor for coupling with bi-, multi- specific or universal engagers;

(iv) deficiency in HLA class I and/or class II molecules;

(v) integrated or non-integrated exogenous polynucleotide encoding one or more of HLA-E, HLA-G, CD16, 41BBL and PDL1 proteins; or introduced expression of one or more of HLA-E, HLA-G, CD16, 41BBL and PDL1 proteins;

(vi) at least one exogenous polynucleotide encodes high affinity CD16 receptor; or at least one exogenous polynucleotide encodes non-cleavable CD16 receptor; or at least one exogenous polynucleotide encodes high affinity and non-cleavable CD16 receptor (hnCD16); or at least one exogenous polynucleotide encodes non-cleavable HLA-E; or at least one exogenous polynucleotide encodes non-cleavable HLA-G;

(vii) B2M null or low, TAP1 null or low, TAP2 null or low; Tapasin null or low; introduced expression of HLA-E or non-cleavable HLA-E; introduced expression of HLA-G or non-cleavable HLA-G; introduced expression of PDL1; high affinity non-cleavable CD16 (hnCD16); a Fc receptor; a T cell receptor (TCR); a chimeric antigen receptor (CAR); one or more suicide genes expressing safety switch protein; a bi-, multi-specific or universal engagers, or any combinations thereof; and

(viii) having improved persistency, increased resistance to immune cells, increased immune-resistance, or increased resistance to T and/or NK cells in comparison to iPSCs without the same genomic engineering; and maintain differentiation potential to non-pluripotent cells having the same functional genomic engineering.

12. A pharmaceutical composition comprising a pharmaceutically acceptable medium and genome-engineered iPSCs according to Claim 10 or 11.

13. Use of a pharmaceutical composition according to Claim 12 in the manufacture of a medicament for the treatment of an autoimmune disorder; a hematological malignancy; a solid tumor; cancer, or an infection associated with HIV, RSV, EBV, CMV, adenovirus, or BK polyomavirus.

14. A composition comprising:

(i) genome-engineered iPSCs according to Claim 10;

and

(ii) a small molecule composition comprising a MEK inhibitor, a GSK3 inhibitor and a ROCK inhibitor,

in which the genome-engineered iPSCs comprise one or more exogenous polynucleotides encoding safety switch proteins, targeting modality, receptors, signaling molecules, transcription factors, pharmaceutically active proteins and peptides, drug target candidates, or proteins promoting engraftment, trafficking, homing, viability, self-

renewal, persistence, and/or survival of the non-pluripotent cell reprogrammed iPSCs or derivative cells thereof; and /or in/dels at one or more endogenous genes associated with targeting modality, receptors, signaling molecules, transcription factors, drug target candidates, immune response regulation and modulation, or proteins suppressing engraftment, trafficking, homing, viability, self-renewal, persistence, and/or survival of the non-pluripotent cell reprogrammed iPSCs or derivative cells thereof.

15. A composition according to Claim 14, further comprising one or more endonuclease capable of selected site recognition for introducing double strand break at selected sites.

16. A composition according to Claim 14, in which the composition has at least one of the following characteristics:

(i) the one or more exogenous polynucleotides is operatively linked to (1) one or more exogenous promoters comprising CMV, EF1α, PGK, CAG, UBC, or other constitutive, inducible, temporal-, tissue-, or cell type- specific promoters; or (2) one or more endogenous promoters comprised in the selected sites comprising AAVS1, CCR5, ROSA26, collagen, HTRP, H11, beta-2 microglobulin, GAPDH, TCR or RUNX1;

(ii) the genome-engineered iPSCs comprise one or more exogenous polynucleotides encoding safety switch proteins selected from caspase, thymidine kinase, cytosine deaminase, modified EGFR, B-cell CD20, and any combinations thereof;

(iii) the genome-engineered iPSCs comprise two or more same or different safety switch protein encoding exogenous polynucleotides integrated in different safe harbor loci comprising AAVS1, CCR5, ROSA26, collagen, HTRP, H11, H11, beta-2 microglobulin, GAPDH, TCR or RUNX1;

(iv) the genome-engineered iPSCs comprise a caspase encoding gene at AAVS1 locus, and a thymidine kinase encoding gene at H11 locus;

(v) the genome-engineered iPSCs comprise one or more in/dels comprising B2M, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, CIITA, RFX5, RFXAP, and any gene in the chromosome 6p21 region; and in which the gene comprising the in/del has no or reduced expression;

(vi) the genome-engineered iPSCs comprise introduced or increased expression in at least one of HLA-E, HLA-G, CD16, 41BBL, CD3, CD4, CD8, CD47, CD113, CD131,

CD137, CD80, PDL1, $A_{2A}R$, CAR, TCR, Fc receptor, an engager, and surface triggering receptor for coupling with bi- or multi- specific or universal engagers;

(vii) the genome-engineered iPSCs are HLA class I and/or class II deficient;

(viii) the genome-engineered iPSCs comprise B2M null or low, TAP1 null or low and/or TAP2 null or low;

(ix) the genome-engineered iPSCs comprise integrated or non-integrated exogenous polynucleotide encoding one or more of HLA-E, HLA-G, CD16, 41BBL and PDL1 proteins; or in which the genome-engineered iPSCs comprise introduced expression of one or more of HLA-E, HLA-G, CD16, 41BBL and PDL1 proteins;

(x) the genome-engineered iPSCs comprise at least one exogenous polynucleotide encodes high affinity CD16 receptor; at least one exogenous polynucleotide encodes non-cleavable CD16 receptor; at least one exogenous polynucleotide encodes high affinity and non-cleavable CD16 receptor (hnCD16); at least one exogenous polynucleotide encodes non-cleavable HLA-E; or at least one exogenous polynucleotide encodes non-cleavable HLA-G;

(xi) the genome-engineered iPSCs have improved persistency, increased resistance to immune cells, or increased immune-resistance; or in which the genome-engineered iPSCs have increased resistance to T and/or NK cells;

(xii) the genome-engineered iPSCs have the potential to differentiate into non-pluripotent cells comprising hematopoietic lineage cells having the same functional targeted genomic editing;

(xiii) the genome-engineered iPSCs have the potential to differentiate into mesodermal cells, CD34 cells, hemogenic endothelium cells, hematopoietic stem and progenitor cells, hematopoietic multipotent progenitor cells, T cell progenitors, NK cell progenitors, T cells, NKT cells, NK cells, or B cells.

17. A cell or a population thereof, in which (i) the cell is an induced pluripotent stem cell (iPSC); (ii) the cell comprises one or more exogenous polynucleotides at a constant region of a T cell receptor (TCR) alpha locus; (iii) the TCR is knocked out; (iv) the one or more exogenous polynucleotides comprise a chimeric antigen receptor (CAR) expressed under the control of an endogenous TCR promoter of the TCR alpha constant region; and (v) the cell is capable of differentiating into an iPSC-derived hematopoietic lineage cell.

18. A cell or population thereof according to Claim 17, in which the cell or population has at least one of the following characteristics: (i) in which the cell is an iPSC obtained from reprogramming a T cell to a pluripotent state (TiPSC) and genomically editing the TiPSC; (ii) in which the cell is an iPSC obtained from reprogramming a T cell comprising the one or more exogenous polynucleotides at the constant region of the TCR alpha locus; (iii) in which the cell further comprises deletion or reduced expression in at least one of B2M, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, CIITA, RFX5, RFXAP and any gene in the chromosome 6p21 region; (iv) in which the cell further comprises introduced or increased expression in at least one of HLA-E, HLA-G, CD16, 41BBL, CD3, CD4, CD8, CD47, CD113, CD131, CD137, CD80, PDL1, $A_{2A}R$, Fc receptor, an engager, and a surface triggering receptor for coupling with bi-, multi- specific or universal engagers; and (v) in which the cell further comprises introduced or increased expression of CD16, in which the CD16 is a high affinity non-cleavable CD16 (hnCD16).

19. A cell or population thereof according to Claim 18, in which the cell or population has at least one of the following characteristics: (i) in which the CAR, when expressed, is at about the same level as an endogenous TCR in a cell in which TCR is not knocked out; (ii) in which the CAR, when expressed, is expressed at about the same developmental stage as an endogenous TCR in a cell in which TCR is not knocked out; (iii) the CAR comprises a CD19 related CAR.

20. A cell or population thereof according to Claim 17, 18 or 19, in which the iPSC-derived hematopoietic lineage cell comprises: a CD34 cell, a hemogenic endothelium cell, a hematopoietic stem and progenitor cell, a hematopoietic multipotent progenitor cell, a T cell progenitor, an NK cell progenitor, a T cell, an NKT cell, an NK cell, or a B cell.