

CLAIMS

1. A method for generating a universal donor cell, the method comprising delivering to a cell:
(a) a first CRISPR nuclease and a first guide RNA (gRNA) targeting a target site within or near a gene that encodes thioredoxin interacting protein (TXNIP); and
(b) a first nucleic acid comprising a nucleotide sequence encoding HLA class I histocompatibility antigen, alpha chain E (HLA-E) that is flanked by (i) a nucleotide sequence homologous with a region located left of the target site of (a) and (ii) a nucleotide sequence homologous with a region located right of the target site of (a),
wherein the first site-directed nuclease cleaves the target site of (a) and the first nucleic acid of (b) is inserted at a site that partially overlaps, completely overlaps, or is contained within, the site of (a), thereby generating a universal donor cell, wherein the universal donor cell has increased cell survival compared to a cell in which the nucleic acid of (b) has not been inserted.
2. The method of claim 1, wherein the nucleotide sequence encoding HLA-E comprises a sequence encoding a HLA-E trimer comprising a B2M signal peptide fused to an HLA-G presentation peptide fused to a B2M membrane protein fused to HLA-E without its signal peptide, optionally wherein the sequence encoding the HLA-E trimer consists essentially of SEQ ID NO: 55.
3. The method of claim 1 or 2, wherein the first gRNA comprises a spacer sequence corresponding to a target sequence consisting of SEQ ID NOS: 15-24.
4. The method of any one of claims 1 to 3, wherein the nucleotide sequence of (b)(i) consists essentially of SEQ ID NO: 25, and the nucleotide sequence of (b)(ii) consists essentially of SEQ ID NO: 32.
5. The method of any one of claims 1 to 4, wherein the method further comprises delivering to the cell:

(c) a second CRISPR nuclease and a second guide RNA (gRNA) targeting a site within or near a gene that encodes beta-2-microglobulin (B2M); and

(d) a second nucleic acid comprising a nucleotide sequence encoding programmed death ligand 1 (PD-L1) that is flanked by (iii) a nucleotide sequence homologous with a region located left of the target site of (c) and a (iv) nucleotide sequence homologous with a region located right of the target site of (c),

wherein the second CRISPR nuclease cleaves the target site of (c) and the second nucleic acid of (d) is inserted at a site that partially overlaps, completely overlaps, or is contained within, the site of (c), wherein the universal donor cell has increased immune evasion and/or cell survival compared to a cell in which the second nucleic acid of (d) has not been inserted.

6. The method of claim 5, wherein the second gRNA comprises a spacer sequence corresponding to a target sequence consisting of SEQ ID NOS: 1-3 or 35-44.

7. The method of claim 5 or 6, wherein the nucleotide sequence of (d)(iii) consists essentially of SEQ ID NO: 7, and the nucleotide sequence of (d)(iv) consists essentially of SEQ ID NO: 13.

8. The method of any one of claims 1 to 7, wherein the first CRISPR nuclease is a Type II Cas9 nuclease or a Type V Cpf1 nuclease and the first CRISPR nuclease is linked to at least one nuclear localization signal; and/or the second CRISPR nuclease is a Type II Cas9 nuclease or a Type V Cpf1 nuclease and the second CRISPR nuclease is linked to at least one nuclear localization signal.

9. The method of any one of claims 1 to 8, wherein the first CRISPR nuclease and the first gRNA are present at a molar ratio of 1:3 and/or the second CRISPR nuclease and the second gRNA are present at a molar ratio of 1:3.

10. The method of any one of claims 1 to 9, wherein the nucleotide sequence encoding HLA-E is operably linked to an exogenous promoter, and/or the nucleotide sequence encoding the PD-L1 is operably linked to an exogenous promoter.

11. The method of claim 10, wherein the exogenous promoter is a constitutive, inducible, temporal-, tissue-, or cell type-specific promoter, optionally wherein the exogenous promoter is a CMV, EF1 α , PGK, CAG, or UBC promoter.
12. The method of any one of claims 1 to 11, wherein the cell is a mammalian cell, optionally wherein the cell is a human cell.
13. The method of any one of claims 1 to 12, wherein the cell is a pluripotent stem cell, an embryonic stem cell, an adult stem cell, an induced pluripotent stem cell, or a hematopoietic stem cell.
14. The method of any one of claims 1 to 13, wherein the cell is a differentiated cell, or a somatic cell and/or the universal donor cell is capable of being differentiated into lineage-restricted progenitor cells or fully differentiated somatic cells.
15. The method of claim 14, wherein the lineage-restricted progenitor cells are pancreatic endoderm progenitors, pancreatic endocrine progenitors, mesenchymal progenitor cells, muscle progenitor cells, blast cells, hematopoietic progenitor cells, or neural progenitor cells and/or the fully differentiated somatic cells are pancreatic beta cells, epithelial cells, endodermal cells, macrophages, hepatocytes, adipocytes, kidney cells, blood cells, cardiomyocytes, or immune system cells.
16. A process for generating universal donor cells, the process comprising:
 - (a) modifying stem cells by inserting a nucleotide sequence encoding programmed death ligand 1 (PD-L1) within or near a gene encoding beta-2 microglobulin (B2M), thereby generating PD-L1 positive cells;
 - (b) enriching for PD-L1 positive cells;
 - (c) modifying the PD-L1 positive cells from (b) by inserting a nucleotide sequence encoding HLA class I histocompatibility antigen, alpha chain E (HLA-E) within

or near a gene encoding thioredoxin interacting protein (TXNIP), thereby generating PD-L1 positive/HLA-E positive cells;

- (d) enriching for PD-L1 positive/HLA-E positive cells;
- (e) single cell sorting to select for PD-L1 positive/HLA-E positive cells;
- (f) characterizing the cells from (e) as universal donor cells; and
- (g) freezing the universal donor cells for long term storage.

17. The process of claim 16, wherein the modifying at (a) comprises delivering to the stem cells (1) a first RNA-guided nuclease and a first guide RNA (gRNA) targeting a target site in a beta-2 microglobulin (B2M) gene locus and (2) a first vector comprising a first nucleic acid, the first nucleic acid comprising (i) a nucleotide sequence homologous with a region located left of the target site in the B2M gene locus, (ii) the nucleotide sequence encoding the PD-L1, and (iii) a nucleotide sequence homologous with a region located right of the target site in the B2M gene locus, wherein the B2M gene locus is cleaved at the target site and the first nucleic acid comprising the nucleotide sequence encoding PD-L1 is inserted into the B2M gene locus, thereby disrupting the B2M gene.

18. The process of claim 16, wherein the modifying at (a) comprises delivering to the stem cells (1) a first ribonucleoprotein (RNP) complex comprising a first RNA-guided nuclease and a first guide RNA (gRNA) targeting a target site in a beta-2 microglobulin (B2M) gene locus and (2) a first vector comprising a first nucleic acid, the first nucleic acid comprising (i) a nucleotide sequence homologous with a region located left of the target site in the B2M gene locus, (ii) the nucleotide sequence encoding PD-L1, and (iii) a nucleotide sequence homologous with a region located right of the target site in the B2M gene locus, wherein the B2M gene locus is cleaved at the target site and the first nucleic acid comprising the nucleotide sequence encoding PD-L1 is inserted into the B2M gene locus, thereby disrupting the B2M gene.

19. The process of any one of claims 16 to 18, wherein the modifying at (c) comprises delivering to the stem cells (1) a second RNA-guided nuclease and a second guide RNA (gRNA) targeting a target site in a thioredoxin interacting protein (TXNIP) gene locus and (2) a second vector comprising a second nucleic acid, the second nucleic acid comprising (i) a nucleotide

sequence homologous with a region located left of the target site in the TXNIP gene locus, (ii) the nucleotide sequence encoding HLA-E, and (iii) a nucleotide sequence homologous with a region located right of the target site in the TXNIP gene locus, wherein the TXNIP gene locus is cleaved at the target site and the second nucleic acid comprising the nucleotide sequence encoding HLA-E is inserted into the TXNIP gene locus, thereby disrupting the TXNIP gene.

20. The process of any one of claims 16 to 18, wherein the modifying at (c) comprises delivering to the PD-L1 positive cells (1) a second ribonucleoprotein (RNP) complex comprising a second RNA-guided nuclease and a second guide RNA (gRNA) targeting a target site in a thioredoxin interacting protein (TXNIP) gene locus and (2) a second vector comprising a second nucleic acid, the second nucleic acid comprising (i) a nucleotide sequence homologous with a region located left of the target site in the TXNIP gene locus, (ii) the nucleotide sequence encoding HLA-E, and (iii) a nucleotide sequence homologous with a region located right of the target site in the TXNIP gene locus, wherein the TXNIP gene locus is cleaved at the target site and the second nucleic acid comprising the nucleotide sequence encoding HLA-E is inserted into the TXNIP gene locus, thereby disrupting the TXNIP gene.