CLAIMS

We claim:

1. A method of editing the genome of a pluripotent stem cell or an iPS cell, the method comprising contacting the cell with:

(i) a nuclease that causes a break within an endogenous coding sequence of an essential gene in the cell, wherein the essential gene encodes a gene product that is required for survival and/or proliferation of the cell, and

(ii) a donor template that comprises a knock-in cassette comprising an exogenous coding sequence for a gene product of interest in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence of the essential gene, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR) of the break, resulting in a genome-edited cell that expresses:

(a) the gene product of interest, and

(b) the gene product encoded by the essential gene that is required for survival and/or proliferation of the cell, or a functional variant thereof.

2. The method of claim 1, wherein, if the knock-in cassette is not integrated into the genome of the cell by homology-directed repair (HDR) in the correct position or orientation, the cell no longer expresses the gene product encoded by the essential gene, or a functional variant thereof.

3. The method of claim 1 or 2, wherein the break is a double-strand break; and/or

wherein the break is located within the last 1000, 500, 400, 300, 200, 100, or 50 base pairs of the endogenous coding sequence of the essential gene; and/or

wherein the break is located within the last exon of the essential gene.

4. The method of any one of claims 1-3, wherein the nuclease is a CRISPR/Cas nuclease and the method further comprises contacting the cell with a guide molecule for the CRISPR/Cas nuclease; or

wherein the nuclease is a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN) or a meganuclease.

5. The method of any one of claims 1-4, wherein the donor template:

(i) is a donor DNA template, optionally wherein the donor DNA template is double-stranded; and optionally,

wherein the donor DNA template is a plasmid, optionally wherein the plasmid has not been linearized; and/or

(ii) comprises homology arms on either side of the knock-in cassette; and optionally,

wherein the homology arms correspond to sequences located on either side of the break in the genome of the cell; and/or

(iii) does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.

6. The method of any one of claims 1-5, wherein the knock-in cassette comprises:

(i) a regulatory element that enables expression of the gene product encoded by the essential gene and the gene product of interest as separate gene products, optionally, wherein at least one of the gene products is a protein and the regulatory element enables expression of that protein separate from the other gene product; and optionally,

wherein the knock-in cassette comprises an IRES or 2A element located between the exogenous coding sequence or partial coding sequence of the essential gene and the exogenous coding sequence for the gene product of interest; and optionally,

wherein the 2A element is a T2A element (EGRGSLLTCGDVEENPGP), a P2A element (ATNFSLLKQAGDVEENPGP), a E2A element (QCTNYALLKLAGDVESNPGP), or an F2A element (VKQTLNFDLLKLAGDVESNPGP); and/or

wherein the knock-in cassette further comprises a sequence encoding a linker peptide upstream of the 2A element; and optionally,

wherein the linker peptide comprises the amino acid sequence GSG; and/or

(ii) a polyadenylation sequence, and optionally a 3' UTR sequence, downstream of the exogenous coding sequence for the gene product of interest, wherein, if a 3'UTR sequence is present, the 3'UTR sequence is positioned 3' of the exogenous coding sequence and 5' of the polyadenylation sequence.

7. The method of any one of claims 1-6, wherein the exogenous partial coding sequence of the essential gene in the knock-in cassette encodes a C-terminal fragment of a protein encoded by the essential gene; and optionally,

wherein the C-terminal fragment is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 15 or 10 amino acids in length; and/or

wherein the C-terminal fragment includes an amino acid sequence that is encoded by a region of the endogenous coding sequence of the essential gene that spans the break.

8. The method of any one of claims 1-7, wherein the exogenous coding sequence or partial coding sequence of the essential gene in the knock-in cassette is less than 100% identical to the corresponding endogenous coding sequence of the essential gene of the cell; and optionally,

wherein the exogenous coding sequence or partial coding sequence of the essential gene in the knock-in cassette has been codon optimized relative to the corresponding endogenous coding sequence of the essential gene of the cell to prevent further binding of the nuclease to the target site, to reduce the likelihood of recombination after integration of the knock-in cassette into the genome of the cell, and/or to increase expression of the gene product of the essential gene and/or the gene product of interest after integration of the knock-in cassette into the genome of the cell.

9. The method of any one of claims 1-8, wherein the essential gene is a housekeeping gene, e.g., a gene listed in Table 3; and/or

wherein the cell is an iPS cell or ES cell and the essential gene is involved in differentiation of iPS or ES cells or expansion of iPS- or ES-derived cells, e.g., a gene listed in Table 4; and optionally, wherein the iPS-derived cells are iPS-derived NK cells or iPS-derived T cells.

10. The method of any one of claims 1-9, wherein the gene product of interest is a chimeric antigen receptor (CAR), a non-naturally occurring variant of FcγRIII (CD16), an interleukin (e.g., interleukin 15 (IL-15), interleukin 15 receptor (IL-15R) or a variant thereof, interleukin 12 (IL-12), interleukin-12 receptor (IL-12R) or a variant thereof), a human leukocyte antigen (e.g., human leukocyte antigen G (HLA-G), human leukocyte antigen E (HLA-E)), leukocyte surface antigen cluster of differentiation 47 (CD47), or any combination of two or more thereof.

11. A genetically modified pluripotent stem cell or iPS cell comprising a genome with an exogenous coding sequence for a gene product of interest in frame with and downstream (3') of a coding sequence of an essential gene, wherein the essential gene encodes a gene product that is required for survival and/or proliferation of the cell.

12. An engineered pluripotent stem cell or iPS cell comprising a genomic modification, wherein the genomic modification comprises an insertion of an exogenous knock-in cassette within an endogenous coding sequence of an essential gene in the cell's genome, wherein the essential gene encodes a gene product that is required for survival and/or proliferation of the cell, wherein the knock-in cassette comprises an exogenous coding sequence for a gene product of interest in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence encoding the gene product of the essential gene, or a functional variant thereof, and wherein the cell expresses the gene product of interest and the gene product encoded by the essential gene that is required for survival and/or proliferation of the cell, or a functional variant thereof.

13. The cell of claim 12, wherein the gene product of interest and the gene product encoded by the essential gene are expressed from the endogenous promoter of the essential gene.

14. The cell of any one of claims 11-13, wherein the cell's genome:

(i) comprises a regulatory element that enables expression of the gene product encoded by the essential gene and the gene product of interest as separate gene products, optionally, wherein at least one of the gene products is a protein and the regulatory element enables expression of that protein separate from the other gene product; and optionally,

wherein the cell's genome comprises an IRES or 2A element located between the coding sequence of the essential gene and the exogenous coding sequence for the gene product of interest; and/or

(ii) comprises a polyadenylation sequence, and optionally a 3' UTR sequence, downstream of the exogenous coding sequence for the gene product of interest, wherein, if a 3'UTR sequence is present, the 3'UTR sequence is positioned 3' of the exogenous coding sequence and 5' of the polyadenylation sequence; and/or

(iii) does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.

15. The cell of any one of claims 11-14, wherein the coding sequence of the essential gene is less than 100% identical to an endogenous coding sequence of the essential gene.

16. The cell of any one of claims 11-15, wherein the essential gene is a housekeeping gene, e.g., a gene listed in Table 3; and/or

wherein the cell is an iPS cell or ES cell and the essential gene is involved in differentiation of iPS or ES cells or expansion of iPS- or ES-derived cells, e.g., a gene listed in Table 4; and optionally, wherein the iPS-derived cells are iPS-derived NK cells or iPS-derived T cells.

17. The cell of any one of claims 11-16, wherein the gene product of interest is a chimeric antigen receptor (CAR), a non-naturally occurring variant of FcγRIII (CD16), interleukin 15 (IL-15), interleukin 15 receptor (IL-15R) or a variant thereof, interleukin 12 (IL-12), interleukin-12 receptor (IL-12R) or a variant thereof, human leukocyte antigen G (HLA-G), human leukocyte antigen E (HLA-E), leukocyte surface antigen cluster of differentiation 47 (CD47), or any combination of two or more thereof.

18. The cell of any one of claims 11-17, for use as a medicament.

19. A cell produced by the method of any one of claims 1-10 or progeny thereof.

20. A population of cells produced by the method of any one of claims 1-10 or progeny thereof.

21. A system for editing the genome of a pluripotent stem cell or an iPS cell, the system comprising the cell, a nuclease that causes a break within an endogenous coding sequence of an essential gene of the cell, wherein the essential gene encodes a gene product that is required for survival and/or proliferation of the cell, and a donor template that comprises a knock-in cassette comprising an exogenous coding sequence for a gene product of interest in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence of the essential gene.

22. The system of claim 21, wherein the break is a double-strand break; and/or

wherein the break is located within the last 1000, 500, 400, 300, 200, 100 or 50 base pairs of the coding sequence of the essential gene; and/or

wherein the break is located within the last exon of the essential gene.

23. The system of claim 21 or 22, wherein the nuclease is a CRISPR/Cas nuclease and the system further comprises a guide molecule for the CRISPR/Cas nuclease; or

wherein the nuclease is a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN) or a meganuclease.

24. The system of any one of claims 21-23, wherein the donor template:

(i) is a donor DNA template, optionally wherein the donor DNA template is double-stranded; and optionally,

wherein the donor DNA template is a plasmid, optionally wherein the plasmid has not been linearized; and/or

(ii) comprises homology arms on either side of the knock-in cassette; and optionally,

wherein the homology arms correspond to sequences located on either side of the break in the genome of the cell; and/or

(iii) does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.

25. The system of any one of claims 21-24, wherein the knock-in cassette comprises:

(i) a regulatory element that enables expression of the gene product encoded by the essential gene and the gene product of interest as separate gene products, optionally, wherein at least one of the

gene products is a protein and the regulatory element enables expression of that protein separate from the other gene product; and optionally,

wherein the knock-in cassette comprises an IRES or 2A element located between the exogenous coding sequence or partial coding sequence of the essential gene and the exogenous coding sequence for the gene product of interest; and/or

(ii) a polyadenylation sequence, and optionally a 3' UTR sequence, downstream of the exogenous coding sequence for the gene product of interest, wherein, if a 3'UTR sequence is present, the 3'UTR sequence is positioned 3' of the exogenous coding sequence and 5' of the polyadenylation sequence.

26. The system of any one of claims 21-25, wherein the exogenous partial coding sequence of the essential gene in the knock-in cassette encodes a C-terminal fragment of a protein encoded by the essential gene; and optionally,

wherein the C-terminal fragment is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 15 or 10 amino acids in length; and/or

wherein the C-terminal fragment includes an amino acid sequence that is encoded by a region of the coding sequence of the essential gene that spans the break.

27. The system of any one of claims 21-26, wherein the exogenous coding sequence or partial coding sequence of the essential gene in the knock-in cassette is less than 100% identical to the corresponding endogenous coding sequence of the essential gene of the cell; and optionally,

wherein the exogenous coding sequence or partial coding sequence of the essential gene in the knock-in cassette has been codon optimized relative to the corresponding endogenous coding sequence of the essential gene of the cell to prevent further binding of a nuclease to the target site, to reduce the likelihood of recombination after integration of the knock-in cassette into the genome of the cell, or to increase expression of the gene product of the essential gene and/or the gene product of interest after integration of the knock-in cassette into the genome of the cell; and optionally,

wherein the exogenous coding sequence or partial coding sequence of the essential gene in the knock-in cassette does not comprise a target site for the nuclease.

28. The system of any one of claims 21-27, wherein the essential gene is a housekeeping gene, e.g., a gene listed in Table 3; and/or

wherein the cell is an iPS cell or ES cell and the essential gene is involved in differentiation of iPS or ES cells or expansion of iPS- or ES-derived cells, e.g., a gene listed in Table 4; and optionally, wherein the iPS-derived cells are iPS-derived NK cells or iPS-derived T cells.

29. The system of any one of claims 21-28, wherein the gene product of interest is a chimeric antigen receptor (CAR), a non-naturally occurring variant of FcγRIII (CD16), interleukin 15 (IL-15), interleukin 15 receptor (IL-15R) or a variant thereof, interleukin 12 (IL-12), interleukin-12 receptor (IL-12R) or a variant thereof, human leukocyte antigen G (HLA-G), human leukocyte antigen E (HLA-E), leukocyte surface antigen cluster of differentiation 47 (CD47), or any combination of two or more thereof.

30. A donor template for use in editing the genome of a pluripotent stem cell or an iPS cell, the donor template comprising a knock-in cassette with an exogenous coding sequence for a gene product of interest in frame with and downstream (3′) of an exogenous coding sequence or partial coding sequence of an essential gene, wherein the essential gene encodes a gene product that is required for survival and/or proliferation of a pluripotent stem cell or an iPS cell.

31. The donor template of claim 30, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR).

32. The donor template of claim 30 or 31, wherein the donor template:

(i) is a donor DNA template, optionally wherein the donor DNA template is double-stranded; and optionally,

wherein the donor DNA template is a plasmid, optionally wherein the plasmid has not been linearized; and/or

(ii) comprises homology arms on either side of the knock-in cassette; and/or

(iii) does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.

33. The donor template of any one of claims 30-32, wherein the knock-in cassette comprises:

(i) a regulatory element that enables expression of the gene product encoded by the essential gene and the gene product of interest as separate gene products, optionally, wherein at least one of the gene products is a protein and the regulatory element enables expression of that protein separate from the other gene product; and optionally,

wherein the knock-in cassette comprises an IRES or 2A element located between the exogenous coding sequence or partial coding sequence of the essential gene and the exogenous coding sequence for the gene product of interest; and/or

(ii) a polyadenylation sequence, and optionally a 3′ UTR sequence, downstream of the exogenous coding sequence for the gene product of interest, wherein, if a 3′UTR sequence is present,

the 3′UTR sequence is positioned 3′ of the exogenous coding sequence and 5′ of the polyadenylation sequence.

34. The donor template of any one of claims 30-33, wherein the exogenous partial coding sequence of the essential gene in the knock-in cassette encodes a C-terminal fragment of a protein encoded by the endogenous coding sequence of the essential gene; and optionally,

wherein the C-terminal fragment is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 15 or 10 amino acids in length.

35. The donor template of any one of claims 30-34, wherein the exogenous coding sequence or partial coding sequence of the essential gene in the knock-in cassette is less than 100% identical to the corresponding endogenous coding sequence of the essential gene; and optionally,

wherein the exogenous coding sequence or partial coding sequence of the essential gene in the knock-in cassette has been codon optimized relative to the corresponding endogenous coding sequence of the essential gene to prevent further binding of a nuclease to the target site, to reduce the likelihood of recombination after integration of the knock-in cassette into a genome of a cell, or to increase expression of the gene product of the essential gene and/or the gene product of interest after integration of the knock-in cassette into a genome of a cell; and optionally,

wherein the exogenous coding sequence or partial coding sequence of the essential gene in the knock-in cassette does not comprise a target site for a nuclease.

36. The donor template of any one of claims 30-35, wherein the essential gene is a housekeeping gene, e.g., a gene listed in Table 3; and/or

wherein the cell is an iPS cell or ES cell and the essential gene is involved in differentiation of iPS or ES cells or expansion of iPS- or ES-derived cells, e.g., a gene listed in Table 4.

37. The donor template of any one of claims 30-36, wherein the gene product of interest is a chimeric antigen receptor (CAR), a non-naturally occurring variant of FcγRIII (CD16), interleukin 15 (IL-15), interleukin 15 receptor (IL-15R) or a variant thereof, interleukin 12 (IL-12), interleukin-12 receptor (IL-12R) or a variant thereof, human leukocyte antigen G (HLA-G), human leukocyte antigen E (HLA-E), leukocyte surface antigen cluster of differentiation 47 (CD47), or any combination of two or more thereof.

38. Use of the cell of any one of claims 11-17 in the manufacture of a medicament for treating cancer.