## CLAIMS

We claim:

~~1. A method of editing the genome of a 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.~~

~~4. The method of any one of claims 1-3, 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.~~

~~5. The method of any one of claims 1-3, wherein the break is located within the last exon of the essential gene.~~

~~6. The method of any one of claims 1-5, 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.~~

~~7. The method of any one of claims 1-5, wherein the nuclease is a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN) or a meganuclease.~~

~~8. The method of any one of claims 1-7, wherein the donor template is a donor DNA template, optionally wherein the donor DNA template is double-stranded.~~

~~9. The method of claim 8, wherein the donor DNA template is a plasmid, optionally wherein the plasmid has not been linearized.~~

~~10. The method of any one of claims 1-9, wherein the donor template comprises homology arms on either side of the knock-in cassette.~~

11. The method of claim 10, wherein the homology arms correspond to sequences located on either side of the break in the genome of the cell.

12. The method of any one of claims 1-11, wherein the knock-in cassette 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein the 2A element is a T2A element (EGRGSLLTCGDVEENPGP), a P2A element (ATNFSLLKQAGDVEENPGP), a E2A element (QCTNYALLKLAGDVESNPGP), or an F2A element (VKQTLNFDLLKLAGDVESNPGP).

15. The method of claim 13 or 14, wherein the knock-in cassette further comprises a sequence encoding a linker peptide upstream of the 2A element.

16. The method of claim 15, wherein the linker peptide comprises the amino acid sequence GSG.

17. The method of any one of claims 1-16, wherein the knock-in cassette 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.

18. The method of any one of claims 1-17, 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.

19. The method of claim 18, wherein the C-terminal fragment is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 15 or 10 amino acids in length.

20. The method of claim 18 or 19, 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.

21. The method of any one of claims 1-20, 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.

22. The method of claim 21, 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.

23. The method of any one of claims 1-22, wherein the essential gene is a housekeeping gene, e.g., a gene listed in Table 3.

24. The method of any one of claims 1-22, 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.

25. The method of claim 24, wherein the iPS-derived cells are iPS-derived NK cells or iPS-derived T cells.

26. The method of any one of claims 1-25, wherein the donor template does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.

27. The method of any one of claims 1-26, 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 CD47 (CD47), or any combination of two or more thereof.

28. A genetically modified 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.

29. An engineered 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, optionally 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.

30. The cell of claim 28 or 29, wherein the cell's genome 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.~~

~~31. The cell of claim 30, 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.~~

~~32. The cell of any one of claims 28-31, wherein the cell's genome 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.~~

~~33. The cell of any one of claims 28-32, wherein the coding sequence of the essential gene is less than 100% identical to an endogenous coding sequence of the essential gene.~~

~~34. The cell of any one of claims 28-33, wherein the essential gene is a housekeeping gene, e.g., a gene listed in Table 3.~~

~~35. The cell of any one of claims 28-33, 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.~~

~~36. The cell of claim 35, wherein the iPS-derived cells are iPS-derived NK cells or iPS-derived T cells.~~

~~37. The cell of any one of claims 28-36, wherein the cell's genome does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.~~

~~38. The cell of any one of claims 28-37, 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 CD47 (CD47), or any combination of two or more thereof.~~

~~39. The cell of any one of claims 28-38, for use as a medicament.~~

~~40. The cell of any one of claims 28-38, for use in the treatment of a disease, disorder, or condition, e.g., a cancer.~~

~~41. A cell, or population of cells, produced by the method of any one of claims 1-27 or progeny thereof.~~

~~42. A system for editing the genome of a 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.

43. The system of claim 42, wherein the break is a double-strand break.

44. The system of claim 42 or 43, 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.

45. The system of any one of claims 42-44, wherein the break is located within the last exon of the essential gene.

46. The system of any one of claims 42-45, wherein the nuclease is a CRISPR/Cas nuclease and the system further comprises a guide molecule for the CRISPR/Cas nuclease.

47. The system of any one of claims 42-45, wherein the nuclease is a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN) or a meganuclease.

48. The system of any one of claims 42-47, wherein the donor template is a donor DNA template, optionally wherein the donor DNA template is double-stranded.

49. The system of claim 48, wherein the donor DNA template is a plasmid, optionally wherein the plasmid has not been linearized.

50. The system of any one of claims 42-49, wherein the donor template comprises homology arms on either side of the knock-in cassette.

51. The system of claim 50, wherein the homology arms correspond to sequences located on either side of the break in the genome of the cell.

52. The system of any one of claims 42-51, wherein the knock-in cassette 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.

53. The system of claim 52, 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.

54. The system of any one of claims 42-53, wherein the knock-in cassette 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.

55. The system of any one of claims 42-54, 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.

56. The system of claim 55, wherein the C-terminal fragment is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 15 or 10 amino acids in length.

~~57. The system of claim 55 or 56, 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.~~

~~58. The system of any one of claims 42-57, 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.~~

~~59. The system of claim 58, 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.~~

~~60. The system of claim 59, 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.~~

~~61. The system of any one of claims 42-60, wherein the essential gene is a housekeeping gene, e.g., a gene listed in Table 3.~~

~~62. The system of any one of claims 42-61, 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.~~

~~63. The system of claim 62, wherein the iPS-derived cells are iPS-derived NK cells or iPS-derived T cells.~~

~~64. The system of any one of claims 42-63, wherein the donor DNA template does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.~~

~~65. The system of any one of claims 42-64, 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 CD47 (CD47), or any combination of two or more thereof.~~

~~66. A 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 the cell.~~

~~67. The donor template of claim 66, for use in editing the genome of a cell by homology-directed repair (HDR)~~

68. The donor template of claim 66 or 67, wherein the donor template is a donor DNA template, optionally wherein the donor DNA template is double-stranded.

69. The donor template of claim 68, wherein the donor DNA template is a plasmid, optionally wherein the plasmid has not been linearized.

70. The donor template of any one of claims 66-69, wherein the donor template comprises homology arms on either side of the knock-in cassette.

71. The donor template of any one of claims 66-70, wherein the knock-in cassette 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.

72. The donor template of claim 71, 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.

73. The donor template of any one of claims 66-72, wherein the knock-in cassette 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.

74. The donor template of any one of claims 66-73, 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.

75. The donor template of claim 74, wherein the C-terminal fragment is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 15 or 10 amino acids in length.

76. The donor template of any one of claims 66-75, 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.

77. The donor template of claim 76, 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.

78. The donor template of claim 77, 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.

79. The donor template of any one of claims 66-78, wherein the essential gene is a housekeeping gene, e.g., a gene listed in Table 3.

80. The donor template of any one of claims 66-79, 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.

81. The donor template of any one of claims 66-80, wherein the donor template does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.

82. The donor template of any one of claims 66-81, 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 CD47 (CD47), or any combination of two or more thereof.

83. A method of generating genetically modified mammalian cells comprising a safety switch comprising:

providing at least one donor nucleic acid construct comprising a genetic payload comprising at least one necessary component of a safety switch

wherein said genetic payload is flanked by a first homologous region (HR) and a second HR, wherein the first and second HRs are essentially homologous to a first genomic region (GR) and a second GR, respectively, wherein the first GR and the second GR are adjacent to and flank a pre-determined genomic position in an exon of an essential gene in a mammalian cell,

providing a gene editing system containing a nuclease that is targeted to the pre-determined genomic position, and

adding the at least one donor nucleic acid construct and the gene editing system into a population of mammalian cells wherein a plurality of the mammalian cells incorporate the genetic payload at the pre-determined genomic position,

wherein a disruption to the essential gene sequence caused by the nuclease is restored upon integration of the HRs and genetic payload.

84. The method of claim 83 wherein each donor nucleic acid construct comprises at least one necessary component of the safety switch.

85. The method of any of claims 83 or 84, wherein each donor nucleic acid construct comprises all of the necessary components of a safety switch.

86. The method of any one of claims 83 – 85 wherein a combination of the donor nucleic acid constructs contain all of the necessary components of a functional safety switch.

87. The method of any one of claims 83 – 86 wherein the necessary components of the safety switch dimerize to produce a functional suicide switch.

88. The method of any one of claims 83 – 87 wherein the genetic payload from a first donor nucleic acid construct is incorporated into a first allele of the essential gene and the genetic payload from a second donor nucleic acid construct is incorporated into a second allele of the essential gene.

89. The method of any one of claims 83 – 88 wherein one or more of the necessary components of the safety switch are incorporated into a first allele of the essential gene and the rest of the necessary components of the safety switch are incorporated into the second allele of the essential gene.

90. The method of any one of claims 83 – 89 wherein activation of the safety switch is triggered by a cellular event, an environmental event or a chemical agent.

91. The method of any of claims 83 – 90 wherein activation of the safety switch induces apoptosis.

92. The method of any one of claims 83 – 91wherein activation of the safety switch inhibits growth of cells that have incorporated all of the necessary components of the safety switch.

93. A population of cells made by the method of any one of claims 83 – 92.

94. The population of cells of claim 93, wherein the cells are pluripotent stem cells (PSCs).

95. The population of cells of claim 93, wherein the cells are induced pluripotent stem cells (iPSCs).

96. A cell from the population of cells of any one of claims 93 – 95 wherein the cell is differentiated into a differentiated cell.

97. The differentiated cell of claim 96, wherein the differentiated cell is selected from:

a cell in the immune system, optionally selected from a T cell, a T cell expressing a chimeric antigen receptor (CAR), a suppressive T cell, a myeloid cell, a dendritic cell, and a macrophage;

a cell in the nervous system, optionally selected from a dopaminergic neuron, a microglia cell, an oligodendrocyte, an astrocyte, a cortical neuron, a spinal or oculomotor neuron, an enteric neuron, a Placode-derived cell, a Schwann cell, and a trigeminal or sensory neuron;

a cell in the ocular system, optionally selected from a retinal pigment epithelial cell, a photoreceptor cone cell, a photoreceptor rod cell, a bipolar cell, and a ganglion cell;

a cell in the cardiovascular system, optionally selected from a cardiomyocyte, an endothelial cell, and a nodal cell; or

a cell in the metabolic system, optionally selected from a hepatocyte, a cholangiocyte, and a pancreatic beta cell.

98. A method of increasing the percentage of cells in the population of cells of claim 93 – 97 that incorporate the genetic payload at the pre-determined genomic position comprising:

creating a first population of mammalian cells comprising cells of any of claims 93 – 97 by providing at least one donor nucleic acid construct comprising a specific genetic payload flanked by a first homologous region (HR) and a second HR, wherein the first and second HRs are essentially homologous to a first genomic region (GR) and a second GR, respectively, wherein the first GR and the second GR are adjacent to and flank a pre-determined genomic position in an exon of an essential gene in a mammalian cell,

providing a gene editing system containing a nuclease that is targeted to the pre-determined genomic position,

providing the at least one donor nucleic acid construct and the gene editing system into the first population of mammalian cells,

culturing the first population of mammalian cells, and

identifying the percentage of surviving cells that comprise the specific genetic payload,

creating a second population of mammalian cells by expanding the surviving cells from the first population of mammalian cells by providing to the surviving cells from the first population of mammalian cells, a gene editing system containing a nuclease that is targeted to the pre-determined genomic position;

optionally reintroducing the at least one donor construct;

culturing the second population of mammalian cells; and

identifying the percentage of surviving cells that comprise the specific exogenous genetic payload,

wherein the percentage of surviving cells from the second population of mammalian cells that comprise the specific exogenous genetic payload is higher than the percentage of surviving cells from the first population of mammalian cells that comprise the specific exogenous genetic payload.

99. The method of claim 98 wherein a plurality of the surviving cells from the first population of mammalian cells that do not comprise the specific genetic payloads are killed during the creation of the second population of mammalian cells.

100. The method of any one of claims 98 or 99 wherein a plurality of surviving cells from the first population of mammalian cells that do not comprise the specific genetic payloads incorporate the specific genetic payloads during the creation of the second population of mammalian cells.

101. The method of any one of claims 98 – 100 wherein the percentage of surviving cells from the second population of mammalian cells that comprise the specific genetic payloads is at least three times larger than the percentage of surviving cells from the first population of mammalian cells that comprise the specific genetic payloads.

102. The method of any one of the preceding claims 98 – 101 wherein the percentage of surviving cells from the second population of mammalian cells that do not comprise the specific

genetic payloads is at least five (5) times lower than the percentage of surviving cells from the first population of mammalian cells that do not comprise the specific genetic payloads.

103. The method of any one of claims 98 – 102 wherein at least one of the donor nucleic acid constructs has a different genetic payload than at least one other donor nucleic acid constructs, and at least a plurality of the second population of mammalian cells incorporate each of the different genetic payloads.

104. The method of any one of claims 98 – 103 wherein the at least one of the HR regions contains at least one mutation that prevents the cutting of the genetic payload at the nuclease cutting site.

105. The method of claims 98 – 104 wherein identifying the percentage of surviving cells is accomplished using flow cytometry.

106. An engineered iPSC comprising a genomic modification, wherein the genomic modification comprises an insertion of an exogenous knock-in cassette within an endogenous coding sequence of a GAPDH gene in the iPSC's genome, wherein the knock-in cassette comprises an exogenous coding sequence for a safety switch in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence encoding GAPDH, or a functional variant thereof, and wherein the iPSC expresses the gene product of interest and GAPDH, or a functional variant thereof, optionally wherein the gene product of interest and the GAPDH are expressed from the endogenous promoter of the GAPDH gene.

107. The iPSC of claim 106, wherein the iPSC's genome comprises a regulatory element that enables expression of GAPDH 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.

108. The iPSC of claim 107, wherein the iPSC's genome comprises an IRES or 2A element located between the coding sequence of the GAPDH gene and the exogenous coding sequence for the gene product of interest.

109. The iPSC of any one of claims 106 – 108, wherein the iPSC's genome 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.

110. The iPSC of any one of claims 106 – 109, wherein the coding sequence of the GAPDH gene is less than 100% identical to an endogenous coding sequence of the GAPDH gene.

111. The iPSC of any one of claims 106 – 110, wherein the iPSC's genome does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.

112. The iPSC of any one of claims 106 – 111, for use as a medicament.

~~113. The iPSC of any one of claims 106 -- 112, for use in the treatment of a disease, disorder, or condition, e.g., a cancer.~~

~~114. A system for editing the genome of an iPSC in a population of iPSCs, the system comprising the population of iPSC, a nuclease that causes a break within an endogenous coding sequence of a GAPDH gene of the iPSC, and a donor template that comprises a knock-in cassette comprising a safety switch in frame with and downstream (3') of an exogenous coding sequence or partial coding sequence of the GAPDH gene.~~

~~115. The system of claim 114, wherein the break is a double-strand break.~~

~~116. The system of any one of claims 114-115, wherein the break is located within the last exon of the GAPDH gene.~~

~~117. The system of any one of claims 114-116, wherein the nuclease is a CRISPR/Cas nuclease and the system further comprises a guide molecule for the CRISPR/Cas nuclease.~~

~~118. The system of any one of claims 114-116, wherein the nuclease is a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN) or a meganuclease.~~

~~119. The system of any one of claims 114-118, wherein the knock-in cassette comprises a regulatory element that enables expression of GAPDH 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.~~

~~120. The system of claim 119, wherein the knock-in cassette comprises an IRES or 2A element located between the exogenous coding sequence or partial coding sequence of the GAPDH gene and the exogenous coding sequence for the gene product of interest.~~

~~121. The system of any one of claims 114 -- 120, wherein the knock-in cassette 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.~~

~~122. The system of any one of claims 114 -- 121, wherein the exogenous coding sequence or partial coding sequence of the GAPDH gene in the knock-in cassette is less than 100% identical to the corresponding endogenous coding sequence of the GAPDH gene of the iPSC.~~

~~123. The system of claim 122, wherein the exogenous coding sequence or partial coding sequence of the GAPDH gene in the knock-in cassette has been codon optimized relative to the corresponding endogenous coding sequence of the GAPDH gene of the iPSC to remove a target site of the DNA-nuclease, and/or to reduce the likelihood of homologous recombination after integration of the knock-in cassette into the genome of the iPSC.~~

~~124. The system of claim 123, wherein the exogenous coding sequence or partial coding sequence of the GAPDH gene in the knock-in cassette does not comprise a target site for the nuclease.~~

~~125. A method of increasing the percentage of genetically modified mammalian cells with a desired genetic payload within a population of mammalian cells comprising:~~

~~creating a first population of mammalian cells by providing at least one donor nucleic acid construct comprising a specific genetic payload flanked by a first homologous region (HR) and a second HR, wherein the first and second HRs are essentially homologous to a first genomic region (GR) and a second GR, respectively, wherein the first GR and the second GR are adjacent to and flank a pre-determined genomic position in an exon of an essential gene in a mammalian cell,~~

~~providing a gene editing system containing a nuclease that is targeted to the pre-determined genomic position,~~

~~providing the at least one donor nucleic acid construct and the gene editing system into the first population of mammalian cells,~~

~~culturing the first population of mammalian cells, and~~

~~identifying the percentage of surviving cells that comprise the specific genetic payload,~~

~~creating a second population of mammalian cells by providing to the surviving cells from the first population of mammalian cells, a gene editing system containing a nuclease that is targeted to the pre-determined genomic position;~~

~~optionally reintroducing the at least one donor construct;~~

~~culturing the second population of mammalian cells; and~~

~~identifying the percentage of surviving cells that comprise the specific exogenous genetic payload,~~

~~wherein the percentage of surviving cells from the second population of mammalian cells that comprise the specific exogenous genetic payload is higher than the percentage of surviving cells from the first population of mammalian cells that comprise the specific exogenous genetic payload.~~

~~126. The method of claim 125 wherein a plurality of the surviving cells from the first population of mammalian cells that do not comprise the specific genetic payloads are killed during the creation of the second population of mammalian cells.~~

~~127. The method any one of claims 125 – 126 wherein a plurality of surviving cells from the first population of mammalian cells that do not comprise the specific genetic payloads incorporate the specific genetic payloads during the creation of the second population of mammalian cells.~~

~~128. The method of any one of claims 125 – 127 wherein the percentage of surviving cells from the second population of mammalian cells that comprise the specific genetic payloads is at least three (3) times larger than the percentage of surviving cells from the first population of mammalian cells that comprise the specific genetic payloads.~~

~~129. The method of any one of claims 125 – 128 wherein at least one of the donor nucleic acid constructs has a different genetic payload than at least one other donor nucleic acid constructs,~~

and at least a plurality of the second population of mammalian cells incorporate each of the different genetic payloads.

130. The method of any one of claims 125 – 129 wherein the percentage of surviving cells from the second population of mammalian cells that do not comprise the specific genetic payloads is at least five (5) times lower than the percentage of surviving cells from the first population of mammalian cells that do not comprise the specific genetic payloads.

131. The method of any one of claims 125 – 130 wherein the at least one of the HR regions contains at least one mutation that prevents the cutting of the genetic payload at the nuclease cutting site.

132. The method of any one of claims 125 – 131 wherein identifying the percentage of surviving cells is accomplished using flow cytometry.

133. A population of cells made by the method of any one of claims 125 – 132.

134. The population of cells of claim 133, wherein the cells are pluripotent stem cells (PSCs).

135. The population of cells of claim 133, wherein the cells are induced pluripotent stem cells (iPSCs).

136. A cell from the population of cells of any one of claims 133 – 135 wherein the cell is differentiated into a differentiated cell.

137. The differentiated cell of claim 136, wherein the differentiated cell is selected from:

a cell in the immune system, optionally selected from a T cell, a T cell expressing a chimeric antigen receptor (CAR), a suppressive T cell, a myeloid cell, a dendritic cell, and a macrophage;

a cell in the nervous system, optionally selected from a dopaminergic neuron, a microglia cell, an oligodendrocyte, an astrocyte, a cortical neuron, a spinal or oculomotor neuron, an enteric neuron, a Placode-derived cell, a Schwann cell, and a trigeminal or sensory neuron;

a cell in the ocular system, optionally selected from a retinal pigment epithelial cell, a photoreceptor cone cell, a photoreceptor rod cell, a bipolar cell, and a ganglion cell;

a cell in the cardiovascular system, optionally selected from a cardiomyocyte, an endothelial cell, and a nodal cell; or

a cell in the metabolic system, optionally selected from a hepatocyte, a cholangiocyte, and a pancreatic beta cell.

138. An iPSC of claim 135, wherein the iPSC's genome comprises a regulatory element that enables expression of GAPDH 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.

~~139. The iPSC of claim 138, wherein the iPSC's genome comprises an IRES or 2A element located between the coding sequence of the GAPDH gene and the exogenous coding sequence for the gene product of interest.~~

~~140. The iPSC of any one of claims 138 – 139, wherein the iPSC's genome 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.~~

~~141. The iPSC of any one of claims 138 – 140, wherein the coding sequence of the GAPDH gene is less than 100% identical to an endogenous coding sequence of the GAPDH gene.~~

~~142. The iPSC of any one of claims 138 – 141, wherein the iPSC's genome does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.~~

~~143. The iPSC of any one of claims 138 – 142, for use as a medicament.~~

~~144. The iPSC of any one of claims 138 – 143, for use in the treatment of a disease, disorder, or condition, e.g., a cancer.~~

~~145~~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.

~~146~~2. The method of claim ~~145~~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.

~~147~~3. The method of claim ~~145~~ 1 or ~~146~~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.

~~148. The method of any one of claims 145-147, 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.~~

~~149. The method of any one of claims 145-147, wherein the break is located within the last exon of the essential gene.~~

~~150~~4. The method of any one of claims ~~145~~1-~~149~~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.

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~~151. The method of any one of claims 145-149, wherein the nuclease is a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN) or a meganuclease.~~

~~152~~5. The method of any one of claims ~~145~~1-~~151~~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.

~~153. The method of claim 152, wherein the donor DNA template is a plasmid, optionally wherein the plasmid has not been linearized.~~

~~154. The method of any one of claims 145-153, wherein the donor template comprises homology arms on either side of the knock-in cassette.~~

~~155. The method of claim 154, wherein the homology arms correspond to sequences located on either side of the break in the genome of the cell.~~

~~156~~6. The method of any one of claims ~~145~~1-~~155~~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.

~~157. The method of claim 156, 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.~~

~~158. The method of claim 157, wherein the 2A element is a T2A element (EGRGSLLTCGDVEENPGP), a P2A element (ATNFSLLKQAGDVEENPGP), a E2A element (QCTNYALLKLAGDVESNPGP), or an F2A element (VKQTLNFDLLKLAGDVESNPGP).~~

~~159. The method of claim 157 or 158, wherein the knock-in cassette further comprises a sequence encoding a linker peptide upstream of the 2A element.~~

~~160. The method of claim 159, wherein the linker peptide comprises the amino acid sequence GSG.~~

~~161. The method of any one of claims 145-160, wherein the knock-in cassette 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.~~

~~162~~7. The method of any one of claims ~~145~~1-~~161~~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.

~~163. The method of claim 162, wherein the C-terminal fragment is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 15 or 10 amino acids in length.~~

~~164. The method of claim 162 or 163, 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.~~

~~165~~8. The method of any one of claims ~~145~~1-~~164~~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.

~~166. The method of claim 165, 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.~~

~~167~~9. The method of any one of claims ~~145~~1-~~166~~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.

~~168. The method of any one of claims 145-166, 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.~~

~~169. The method of claim 168, wherein the iPS-derived cells are iPS-derived NK cells or iPS-derived T cells.~~

~~170. The method of any one of claims 145-169, wherein the donor template does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.~~

~~171~~10. The method of any one of claims ~~145-170~~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 ~~CD~~47 (CD47), or any combination of two or more thereof.

~~172~~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.

~~173~~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. ~~, optionally~~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.

~~174~~14. The cell of any one of claims ~~172~~ 11-13~~or 173~~, 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.

~~175. The cell of claim 174, 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.~~

~~176. The cell of any one of claims 172-175, wherein the cell's genome 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.~~

~~177~~15. The cell of any one of claims ~~172~~11-~~176~~14, wherein the coding sequence of the essential gene is less than 100% identical to an endogenous coding sequence of the essential gene.

~~178~~16. The cell of any one of claims ~~172~~11-~~177~~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.

~~179. The cell of any one of claims 172-177, 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.~~

~~180. The cell of claim 179, wherein the iPS-derived cells are iPS-derived NK cells or iPS-derived T cells.~~

~~181. The cell of any one of claims 172-180, wherein the cell's genome does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.~~

~~182~~17. The cell of any one of claims ~~172~~11-~~181~~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 ~~CD~~47 (CD47), or any combination of two or more thereof.

~~183~~18. The cell of any one of claims ~~172-182~~11-17, for use as a medicament.

~~184. The cell of any one of claims 172-182, for use in the treatment of a disease, disorder, or condition, e.g., a cancer.~~

~~185~~19. A cell~~, or population of cells,~~ produced by the method of any one of claims ~~145~~1-~~171~~10 or progeny thereof.

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

~~186~~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.

~~187~~22. The system of claim ~~186~~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.

~~188. The system of claim 186 or 187, 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.~~

~~189. The system of any one of claims 186-188, wherein the break is located within the last exon of the essential gene.~~

~~190~~23. The system of ~~any one of~~ claim~~s~~ ~~186~~21 or ~~189~~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.

~~191. The system of any one of claims 186-189, wherein the nuclease is a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN) or a meganuclease.~~

~~192~~24. The system of any one of claims ~~186~~21-~~191~~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.

~~193. The system of claim 192, wherein the donor DNA template is a plasmid, optionally wherein the plasmid has not been linearized.~~

~~194. The system of any one of claims 186-193, wherein the donor template comprises homology arms on either side of the knock-in cassette.~~

~~195. The system of claim 194, wherein the homology arms correspond to sequences located on either side of the break in the genome of the cell.~~

~~196~~25. The system of any one of claims ~~186~~21-~~195~~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.

~~197. The system of claim 196, 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.~~

~~198. The system of any one of claims 186-197, wherein the knock-in cassette 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.~~

~~199~~26. The system of any one of claims ~~186~~21-~~198~~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.

~~200. The system of claim 199, wherein the C-terminal fragment is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 15 or 10 amino acids in length.~~

~~201. The system of claim 199 or 200, 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.~~

~~202~~27. The system of any one of claims ~~186~~21-~~201~~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.

~~203. The system of claim 202, 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.~~

~~204. The system of claim 203, 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.~~

~~205~~28. The system of any one of claims ~~186~~21-~~204~~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.

~~206. The system of any one of claims 186-205, 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.~~

~~207. The system of claim 206, wherein the iPS-derived cells are iPS-derived NK cells or iPS-derived T cells.~~

~~208. The system of any one of claims 186-207, wherein the donor DNA template does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.~~

~~209~~29. The system of any one of claims ~~186~~21-~~208~~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 ~~CD~~47 (CD47), or any combination of two or more thereof.

~~210~~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.

~~211~~31. The donor template of claim ~~210~~30, wherein the knock-in cassette is integrated into the genome of the cell by homology-directed repair (HDR).

~~212~~32. The donor template of claim ~~210~~ 30 or ~~211~~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.

~~213. The donor template of any one of claims 210-212, wherein the donor DNA template is a plasmid, optionally wherein the plasmid has not been linearized.~~

~~214. The donor template of any one of claims 210-213, wherein the donor template comprises homology arms on either side of the knock-in cassette.~~

~~215~~33. The donor template of any one of claims ~~210~~30-~~214~~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.

~~216. The donor template of claim 215, 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.~~

~~217. The donor template of any one of claims 210-216, wherein the knock-in cassette 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.~~

~~218~~34. The donor template of any one of claims ~~210~~30-~~217~~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.

~~219. The donor template of claim 218, wherein the C-terminal fragment is less than 500, 250, 150, 125, 100, 75, 50, 25, 20, 15 or 10 amino acids in length.~~

~~220~~35. The donor template of any one of claims ~~210~~30-~~219~~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.

~~221. The donor template of claim 220, 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.~~

~~222. The donor template of claim 221, 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.~~

~~223~~36. The donor template of any one of claims ~~210~~30-~~222~~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.

~~224. The donor template of any one of claims 210-223, 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.~~

~~225. The donor template of any one of claims 210-224, wherein the donor template does not comprise a reporter gene, e.g., a fluorescent reporter gene or an antibiotic resistance gene.~~

~~226~~37. The donor template of any one of claims ~~210~~30-~~225~~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 ~~CD~~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.