CLAIMS

~~1. An enucleated cell engineered to stimulate an immune killer cell, wherein the enucleated cell comprises at least a first exogenous stimulatory polypeptide and a second exogenous stimulatory polypeptide, wherein the exogenous stimulatory polypeptides are sufficient to stimulate the immune killer cell.~~

~~2. The enucleated cell of claim 1, wherein the immune killer cell is a natural killer (NK) cell.~~

~~3. The enucleated cell of claim 1, wherein the immune killer cell is a CD8+ T cell.~~

~~4. The enucleated cell of claim 1, wherein the enucleated cell comprises at least a first exogenous stimulatory polypeptide, a second exogenous stimulatory polypeptide and a third exogenous stimulatory polypeptide.~~

~~5. The enucleated cell of any one of claims 1-4, wherein at least one of the exogenous stimulatory polypeptides comprises a polypeptide selected from the group consisting of: IL-1, IL-2, IL-12, IL-15, IL-15/IL-15RA fusion, IL-18, IL-21, interferon alpha (IFNα), 4-1BBL, Poliovirus Receptor (PVR/CD155), CD48, human leukocyte antigen (HLA)-A, HLA-C, HLA-G, heparan sulfate (HS), HLA-E, CpG, Immunoglobulin G (IgG), MHC class I chain-related proteins (MIC), B7-H6, NkP44L, Nectin2, NK-T-B-antigen (NTBA), activation-induced C-type lectin (AICL), and insulin-like growth factor 1 (IGF-1).~~

~~6. The enucleated cell of any one of claims 1-4, wherein at least one of the exogenous stimulatory polypeptides comprises IL-15/IL-15RA fusion.~~

~~7. The enucleated cell of any one of claims 1-4, wherein at least one of the exogenous stimulatory polypeptides comprises IL-12.~~

~~8. The enucleated cell of any one of claims 1-4, wherein at least one of the exogenous stimulatory polypeptides comprises 4-1BBL.~~

9. The enucleated cell of claim 5, wherein the MIC protein is selected from the group consisting of: MHC class I chain-related protein A (MICA), MHC class I chain-related protein B (MICB) and UL16 binding proteins (ULBP).

10. The enucleated cell of any one of claims 1-4, wherein at least one of the exogenous stimulatory polypeptides comprises a polypeptide selected from the group consisting of: IL-15/IL-15RA fusion, MICA, MICB, and insulin-like growth factor 1 (IGF-1).

11. The enucleated cell of any one of claims 1-4, wherein the first exogenous stimulatory polypeptide comprises IL-15/IL-15RA and the second exogenous stimulatory polypeptide comprises a polypeptide selected from the group consisting of: IL-1, IL-2, IL-12, IL-18, IL-21, 4-1BBL, IFNα, MICA, MICB, PVR and CD48.

12. The enucleated cell of any one of claims 1-4, wherein the first exogenous stimulatory polypeptide comprises IL-15/IL-15RA and the second exogenous stimulatory polypeptide comprises 4-1BBL.

13. The enucleated cell of any one of claims 1-4, wherein the first exogenous stimulatory polypeptide comprises IL-15/IL-15RA and the second exogenous stimulatory polypeptide comprises IL-12.

14. The enucleated cell of any one of claims 1-4, wherein the first exogenous stimulatory polypeptide comprises IL-12 and the second exogenous stimulatory polypeptide comprises 4-1BBL.

15. The enucleated cell of any one of claims 1-4, wherein the first exogenous stimulatory polypeptide and the second exogenous stimulatory polypeptide are selected from the group consisting of: IL-18 and IL-12, IL-18 and IL-21, IL-12 and 4-1BBL, IL-12 and IL-15/IL-15RA fusion, and 4-1BBL and IL-15/IL-15RA fusion.

16. The enucleated cell of claim 4, wherein the first exogenous stimulatory polypeptide comprises IL-12, the second exogenous polypeptide comprises IL-18 and the third exogenous stimulatory polypeptide comprises IL15/IL-15RA fusion.

17. The enucleated cell of claim 4, wherein the first exogenous stimulatory polypeptide comprises IL-12, the second exogenous polypeptide comprises IL-18 and the third exogenous stimulatory polypeptide comprises IL-15.

18. The enucleated cell of any one of claims 1-4, wherein at least one exogenous polypeptide is present at a copy number of greater than 10^4, 10^5, or 10^6.

19. The enucleated cell of any one of claims 1-4, wherein the first exogenous stimulatory polypeptide is present at a copy number of no more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% greater, or no more than 2, 5, 10, 20, 50, 100, 200, 500, or 1000 times greater than the copy number of the second exogenous stimulatory polypeptide.

20. The enucleated cell of any one of claims 1-4, wherein the second exogenous stimulatory polypeptide is present at a copy number of no more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% greater, or no more than 2, 5, 10, 20, 50, 100, 200, 500, or 1000 times greater than the copy number of the first exogenous stimulatory polypeptide.

21. The enucleated cell of any one of claims 1-4, wherein the first exogenous stimulatory polypeptide and the second exogenous stimulatory polypeptide have an abundance ratio of about 1: 1, from about 2: 1 to 1:2, from about 5: 1 to 1:5, from about 10: 1 to 1: 10, from about 20: 1 to 1:20, from about 50: 1 to 1:50, or from about 100: 1 to 1 :100 by weight or by copy number.

22. The enucleated cell of any one of claims 1-4, wherein the first and the second exogenous stimulatory polypeptides are present as a fusion polypeptide.

~~23. The enucleated cell of claim 4, wherein the first, the second and the third exogenous stimulatory polypeptides are present as a fusion polypeptide.~~

~~24. The enucleated cell of any one of claims 1-23, wherein at least one or more exogenous stimulatory polypeptides are present at the surface of the engineered enucleated cell.~~

~~25. The enucleated cell of any one of claims 1-24, wherein at least one or more exogenous stimulatory polypeptides further comprise a transmembrane domain.~~

~~26. The enucleated cell of claim 25, wherein the transmembrane domain comprises glycophorin A (GPA) or a transmembrane portion thereof.~~

~~27. The enucleated cell of claim 25, wherein the transmembrane domain comprises small integral membrane protein 1 (SMIM1), or a transmembrane portion thereof.~~

~~28. The enucleated cell of any one of claims 1-27, wherein the enucleated cell is capable of activating an NK cell.~~

~~29. The enucleated cell of any one of claims 1-27, wherein the enucleated cell is capable of expanding an NK cell.~~

~~30. The enucleated cell of claim 28 or 29, wherein the NK cell is a memory-like NK cell.~~

~~31. The enucleated cell of any one of claims 1-27, wherein the enucleated cell is capable of activating a CD8+ T-cell.~~

~~32. The enucleated cell of any one of claims 1-27, wherein the enucleated cell is capable of expanding a CD8+ T-cell.~~

33. The enucleated cell of claim 31 or 32, wherein the CD8+ T cell is a memory T cell.

34. The enucleated cell of any one of claims 1-32, which is an erythroid cell.

35. The enucleated cell of claim 34, wherein the erythroid cell is a reticulocyte.

36. The enucleated cell of claim 34, wherein the erythroid cell is an erythrocyte.

37. An engineered enucleated cell comprising a first exogenous stimulatory polypeptide, wherein the first exogenous stimulatory polypeptide comprises an interleukin-15 (IL-15) polypeptide, or a fragment thereof, and an extracellular portion of an interleukin-15 receptor alpha (IL-15RA) polypeptide, or a fragment thereof.

38. The engineered enucleated cell of claim 37, wherein the IL-15 polypeptide and the extracellular portion of an IL-15RA polypeptide are present as a complex.

39. The engineered enucleated cell of claim 37 or 38, wherein the first exogenous stimulatory polypeptide comprises a fusion polypeptide.

40. The engineered enucleated cell of any one of claims 37-39, wherein the IL-15 polypeptide, or a fragment thereof, is linked to the extracellular portion of the IL-15RA polypeptide, or a fragment thereof, by a linker.

41. The engineered enucleated cell of claim 40, wherein the linker comprises GGGGS (SEQ ID NO: 11).

42. The engineered enucleated cell of claim 40 or 41, wherein the linker comprises a $(GGGGS)_3$ linker (SEQ ID NO: 12).

43. The engineered enucleated cell of any one of claims 39-42, wherein the fusion polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO. 1.

44. The engineered enucleated cell of any one of claims 39-42, wherein the fusion polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO. 29 or SEQ ID NO. 37.

45. The engineered enucleated cell of claim 37, wherein the first exogenous stimulatory polypeptide comprises an IL-15 polypeptide, or a fragment thereof, and an IL-15 receptor alpha sushi-binding domain.

46. The engineered enucleated cell of claim 45, wherein the IL-15 polypeptide, or a fragment thereof, and the IL-15 receptor alpha sushi-binding domain are present as a complex.

47. The engineered enucleated cell of claim 45 or 46, wherein the IL-15 polypeptide, or a fragment thereof, and the IL-15 receptor alpha sushi-binding domain are present as a fusion polypeptide.

48. The engineered enucleated cell of any one of claims 45-47, wherein the IL-15 polypeptide, or a fragment thereof, is linked to the IL-15 receptor alpha sushi-binding domain by a linker.

49. The engineered enucleated cell of claim 48, wherein the linker comprises GGGGS (SEQ ID NO: 11).

50. The engineered enucleated cell of claim 48 or 49, wherein the linker comprises a $(GGGGS)_3$ linker (SEQ ID NO: 12).

51. The engineered enucleated cell of claim 47, wherein the fusion polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO. 2.

52. The engineered enucleated cell of claim 47, wherein the fusion polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO. 31.

53. The engineered enucleated cell of claim 37, further comprising a second exogenous stimulatory polypeptide.

54. The engineered enucleated cell of claim 53, wherein the second exogenous stimulatory polypeptide comprises 4-1BBL.

55. The engineered enucleated cell of claim 54, wherein the second exogenous stimulatory polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO. 41.

56. The engineered enucleated cell of claim 54, wherein the second exogenous stimulatory polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO. 43.

57. The engineered enucleated cell of claim 53, wherein the second exogenous stimulatory polypeptide comprises IL-12.

58. The engineered enucleated cell of claim 57, wherein the second exogenous stimulatory polypeptide comprises a fusion polypeptide comprising the p40 and p35 subunits of IL-12.

59. The engineered enucleated cell of claim 57 or 58, wherein the second exogenous stimulatory polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO. 37.

60. The engineered enucleated cell of claim 57 or 58, wherein the second exogenous stimulatory polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO. 55.

61. The engineered enucleated cell of any one of claims 37-60, further comprising one or more exogenous stimulatory polypeptides selected from the group consisting of: IL-1, IL-2, IL-12, IL-18, IL-21, interferon alpha (IFNα), Poliovirus Receptor (PVR/CD155), CD48, human leukocyte antigen (HLA)-A, HLA-C, HLA-G, heparan sulfate (HS), HLA-E, CpG, Immunoglobulin G (IgG), UL16 binding proteins (ULBP), MHC class I chain-related proteins (MIC), B7-H6, NkP44L, Nectin2, NK-T-B antigen (NTBA), activation-induced C-type lectin (AICL) and insulin-like growth factor 1 (IGF-1).

62. The engineered enucleated cell of claim 61, wherein the MIC protein is MHC class I chain-related protein A (MICA) or MHC class I chain-related protein B (MICB).

63. The engineered enucleated cell of any one of claims 37-62, wherein one or more of the exogenous stimulatory polypeptides are present at the surface of the engineered enucleated cell.

64. The engineered enucleated cell of any one of claims 37-63, wherein the exogenous stimulatory polypeptides are sufficient to stimulate an immune killer cell.

65. The engineered enucleated cell of any one of claims 37-64, wherein the first exogenous stimulatory polypeptide is present at a copy number of greater than 10^4, 10^5, or 10^6.

66. The engineered enucleated cell of any one of claims 53-60, wherein the first exogenous stimulatory polypeptide is present at a copy number of no more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% greater, or no more than 2, 5, 10, 20, 50, 100, 200, 500, or 1000 times greater than the copy number of the second exogenous stimulatory polypeptide.

67. The engineered enucleated cell of any one of claims 53-60, wherein the second exogenous stimulatory polypeptide is present at a copy number of no more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% greater, or no more than 2, 5, 10, 20, 50, 100, 200, 500, or 1000 times greater than the copy number of the first exogenous stimulatory polypeptide.

68. The engineered enucleated cell of any one of claims 53-60, wherein the first exogenous stimulatory polypeptide and the second exogenous stimulatory polypeptide have an abundance ratio of about 1: 1, from about 2: 1 to 1:2, from about 5: 1 to 1:5, from about 10: 1 to 1: 10, from about 20: 1 to 1:20, from about 50: 1 to 1:50, or from about 100: 1 to 1 : 100 by weight or by copy number.

69. The engineered enucleated cell of claim 37, further comprising a second exogenous polypeptide and a third exogenous stimulatory polypeptide.

70. The engineered enucleated cell of any one of claims 53-69, wherein the engineered enucleated cell comprises two exogenous stimulatory polypeptides and the two exogenous stimulatory polypeptides are present as a fusion polypeptide.

71. The engineered enucleated cell of any one of claims 53-69, wherein the engineered enucleated cell comprises three exogenous stimulatory polypeptides and the three exogenous stimulatory polypeptides are present as a fusion polypeptide.

72. The engineered enucleated cell of any one of claims 37-71, wherein one or more of the exogenous stimulatory polypeptides further comprise a transmembrane domain.

73. The engineered enucleated cell of claim 72, wherein the transmembrane domain comprises glycophorin A (GPA) or a transmembrane portion thereof.

74. The enucleated cell of claim 72, wherein the transmembrane domain comprises small integral membrane protein 1 (SMIM1), or a transmembrane portion thereof.

75. The engineered enucleated cell of any one of claims 37-74, wherein the engineered enucleated cell is capable of stimulating an immune cell.

~~76. The engineered enucleated cell of claim 75, wherein the immune cell is a killer immune cell.~~

~~77. The engineered enucleated cell of claim 76, wherein the killer immune cell is a natural killer (NK) cell.~~

~~78. The engineered enucleated cell of claim 77, wherein the NK cell is a memory-like NK cell.~~

~~79. The engineered enucleated cell of claim 76, wherein the killer immune cell is a CD8+ T-cell.~~

~~80. The engineered enucleated cell of claim 79, wherein the CD8+ T-cell is a memory T-cell.~~

~~81. The engineered enucleated cell of claim 75, wherein stimulating the immune cell comprises activating the immune cell.~~

~~82. The engineered enucleated cell of claim 75, wherein stimulating the immune cell comprises expanding the immune cell.~~

~~83. The engineered enucleated cell of any one of claims 37-82, which is an erythroid cell.~~

~~84. The engineered enucleated cell of claim 83, wherein the erythroid cell is a reticulocyte.~~

~~85. The engineered enucleated cell of claim 83, wherein the erythroid cell is an erythrocyte.~~

86. An engineered enucleated cell comprising at least a first exogenous stimulatory polypeptide comprising a polypeptide selected from the group consisting of: MHC class I chain-related protein A (MICA), MHC class I chain-related protein B (MICB), and insulin-like growth factor 1 (IGF-1).

87. The engineered enucleated cell of claim 86, wherein the enucleated cell further comprises a second exogenous polypeptide.

88. The engineered enucleated cell of claim 87, wherein the second exogenous stimulatory polypeptide comprises a polypeptide selected from the group consisting of: IL-1, IL-2, IL-12, IL-15, IL-15/IL-15RA fusion, IL-18, IL-21, interferon alpha (IFNα), 4-1BBL, Poliovirus Receptor (PVR/CD155), CD48, HLA-A, HLA-C, HLA-G, heparan sulfate (HS), HLA-E, CpG, IgG, UL16 binding proteins (ULBP), MHC class I chain-related polypeptide (MIC), B7-H6, NkP44L, Nectin2, NK-T-B antigen (NTBA), activation-induced C-type lectin (AICL) and insulin-like growth factor 1 (IGF-1).

89. The engineered enucleated cell of any one of claims 86-88, wherein one or more of the exogenous stimulatory polypeptides are present at the surface of the engineered enucleated cell.

90. The engineered enucleated cell of any one of claims 86-89, wherein the exogenous stimulatory polypeptides are sufficient to stimulate an immune killer cell.

91. The engineered enucleated cell of claim 86, wherein the exogenous stimulatory polypeptide is present at a copy number of greater than 10^4, 10^5, or 10^6.

92. The engineered enucleated cell of claim 87 or 88, wherein the first exogenous stimulatory polypeptide is present at a copy number of no more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% greater, or no more than 2, 5, 10, 20, 50, 100, 200, 500, or 1000 times greater than the copy number of the second exogenous stimulatory polypeptide.

93. The engineered enucleated cell of claim 87 or 88, wherein the second exogenous stimulatory polypeptide is present at a copy number of no more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% greater, or no more than 2, 5, 10, 20, 50, 100, 200, 500, or 1000 times greater than the copy number of the first exogenous stimulatory polypeptide.

94. The engineered enucleated cell of claim 87 or 88, wherein the first exogenous stimulatory polypeptide and the second exogenous stimulatory polypeptide have an abundance ratio of about 1: 1, from about 2: 1 to 1:2, from about 5: 1 to 1:5, from about 10: 1 to 1: 10, from about 20:1 to 1:20, from about 50:1 to 1:50, or from about 100:1 to 1:100 by weight or by copy number.

95. The engineered enucleated cell of claim 87, wherein the enucleated cell further comprises a third exogenous stimulatory polypeptide.

96. The engineered enucleated cell of any one of claims 87-94, wherein the engineered enucleated cell comprises two exogenous stimulatory polypeptides and the two exogenous stimulatory polypeptides are present as a fusion polypeptide.

97. The engineered enucleated cell of any one of claims 87-95, wherein the engineered enucleated cell comprises three exogenous stimulatory polypeptides and the three exogenous stimulatory polypeptides are present as a fusion polypeptide.

98. The engineered enucleated cell of any one of claims 86-97, wherein one or more of the exogenous stimulatory polypeptides comprises a transmembrane domain.

99. The engineered enucleated cell of claim 98, wherein the transmembrane domain comprises glycophorin A (GPA) or a transmembrane portion thereof.

100. The engineered enucleated cell of claim 98, wherein the transmembrane domain comprises small integral membrane protein 1 (SMIM1), or a transmembrane portion thereof.

~~101. The engineered enucleated cell of any one of claims 86-100, wherein the engineered enucleated cell is capable of stimulating an immune cell.~~

~~102. The engineered enucleated cell of claim 101, wherein the immune cell is a killer immune cell.~~

~~103. The engineered enucleated cell of claim 102, wherein the killer immune cell is a natural killer (NK) cell.~~

~~104. The engineered enucleated cell of claim 103, wherein the NK cell is a memory-like NK cell.~~

~~105. The engineered enucleated cell of claim 102, wherein the killer immune cell is a CD8+ T-cell.~~

~~106. The engineered enucleated cell of claim 105, wherein the CD8+ T-cell is a memory T-cell.~~

~~107. The engineered enucleated cell of claim 101, wherein stimulating the immune cell comprises activating the immune cell.~~

~~108. The engineered enucleated cell of claim 101, wherein stimulating the immune cell comprises expanding the immune cell.~~

~~109. An method of stimulating an immune killer cell, comprising contacting the immune killer cell with the engineered enucleated cell of any one of claims 1-108, in an amount effective to stimulate the immune killer cell.~~

~~110. The method of claim 109, wherein the immune killer cell is an NK cell.~~

~~111. The method of claim 109, wherein the immune killer cell is a CD8+ T-cell.~~

~~112. The method of claim 110, wherein the stimulating comprises activating the NK cell.~~

~~113. The method of claim 110, wherein the stimulating comprises expanding the NK cell.~~

~~114. The method of claim 111, wherein the stimulating comprises activating the CD8+ T-cell.~~

~~115. The method of claim 111, wherein the stimulating comprises expanding the CD8+ T-cell.~~

~~116. The method of claim 111, wherein the CD8+ T-cell is a memory T-cell.~~

~~117. The method of any one of claims 109-116, wherein the contacting is performed *in vivo*.~~

~~118. The method of any one of claims 109-116, wherein the contacting is performed *ex vivo*.~~

~~119. The method of any one of claims 109-116, wherein the contacting is performed *in vitro*.~~

~~120. The method of claim 109, wherein the method further comprises administering the engineered enucleated cell to a subject in need of immune killer cell activation.~~

~~121. The method of claim 120, wherein the subject has cancer.~~

~~122. The method of claim 121, wherein the cancer is characterized by low MHC class I presentation.~~

123. The method of claim 121, wherein the cancer comprises a PD-1-responsive tumor.

124. The method of claim 121, wherein the cancer comprises tumors with a high mutational burden.

125. The method of claim 122, wherein the subject is being treated with a chemotherapeutic agent that decreases MHC class I presentation.

126. The method of claim 121, wherein the cancer is selected from lung cancer, hepatocellular cancer, melanoma, and lymphoma.

127. The method of claim 121, wherein the cancer comprises lymphoma, and the lymphoma comprises Hodgkin's lymphoma or non-Hodgkin's lymphoma.

128. The method of claim 120, wherein the subject has an infectious disease.

129. The method of claim 128, wherein the infectious diseases is caused by a viral infection.

130. The method of claim 129, wherein the viral infection is characterized by low MHC class I presentation.

131. The method of claim 129, wherein the viral infection is caused by a virus selected from the group consisting of: adenovirus, Epstein barr virus (EBV), hepatitis B virus (HBV), tuberculosis, human immunodeficiency virus (HIV), herpes simplex virus (HSV), papilloma virus, and cytomegalovirus.

132. A method of treating a cancer in a subject, comprising administering to the subject the engineered enucleated cell of any one of claims 1-108, in an amount effective to treat the cancer in the subject.

~~133. The method of claim 132, wherein the cancer comprises low MHC class I presentation.~~

~~134. The method of claim 132, wherein the cancer comprises a PD-1 responsive tumor.~~

~~135. The method of claim 132, wherein the cancer comprises a tumor with a high mutational burden.~~

~~136. The method of claim 132, wherein the cancer is selected from lung cancer, hepatocellular cancer, melanoma, and lymphoma.~~

~~137. The method of claim 132, wherein the cancer comprises lymphoma, and the lymphoma is selected from Hodgkin's Lymphoma or non-Hodgkin's lymphoma.~~

~~138. A method of treating an infectious disease in a subject, comprising administering to the subject the engineered enucleated cell of any one of claims 1-108, in an amount effective to treat the infectious disease in the subject.~~

~~139. The method of claim 138, wherein the infectious diseases is caused by a viral infection.~~

~~140. The method of claim 139, wherein the viral infection is characterized by down-regulation of MHC I presentation.~~

~~141. The method of claim 139, wherein the viral infection is caused by a virus selected from adenovirus, Epstein-barr virus (EBV), hepatitis B virus (HBV), tuberculosis, human immunodeficiency virus (HIV), herpes simplex virus (HSV), papilloma virus and cytomegalovirus.~~

~~142. An enucleated erythroid cell engineered to stimulate an immune cell, wherein the immune cell is a killer cell, comprising two or more exogenous stimulatory polypeptides at the surface of the engineered enucleated cell, wherein the two or more exogenous stimulatory polypeptides are sufficient to stimulate the immune cell, produced by a process comprising:~~

introducing two or more exogenous nucleic acids, each encoding one of the exogenous stimulatory polypeptides, into a nucleated erythroid cell; and

culturing the nucleated erythroid cell under conditions suitable for enucleation of the nucleated erythroid cell and for production of the two or more exogenous stimulatory polypeptides.

143. An engineered enucleated erythroid cell, comprising a first exogenous stimulatory polypeptide, wherein the first exogenous stimulatory polypeptide comprises an IL-15 polypeptide, or a fragment thereof, linked to the extracellular portion of an IL-15RA polypeptide, or a fragment thereof, by a linker to form an IL-15/IL-15RA fusion, produced by a process comprising:

introducing an exogenous nucleic acid encoding the IL-15/IL-15RA fusion into a nucleated erythroid cell; and

culturing the nucleated erythroid cell under conditions suitable for enucleation of the nucleated erythroid cell and for production of the IL-15/IL-15RA fusion.

144. An engineered enucleated erythroid cell, comprising:

i. a first exogenous stimulatory polypeptide, wherein the first exogenous stimulatory polypeptide comprises an IL-15 polypeptide, or a fragment thereof, linked to the extracellular portion of an IL-15RA polypeptide, or a fragment thereof, by a linker to form an IL-15/IL-15RA fusion,; and

ii. a second exogenous stimulatory polypeptide, wherein the second exogenous stimulatory polypeptide comprises 4-1BBL, or a fragment thereof,

wherein the cell is produced by a process comprising:

i. introducing a first exogenous nucleic acid encoding the first exogenous stimulatory polypeptide into a nucleated erythroid cell;

ii. introducing a second exogenous nucleic acid encoding the second exogenous stimulatory polypeptide into the nucleated erythroid cell; and

iii. culturing the nucleated erythroid cell under conditions suitable for enucleation of the nucleated erythroid cell and for production of the first exogenous stimulatory polypeptide and the second exogenous stimulatory polypeptide.

~~145. An engineered enucleated erythroid cell, comprising:~~

~~i. a first exogenous stimulatory polypeptide, wherein the first exogenous stimulatory polypeptide comprises an IL-15 polypeptide, or a fragment thereof, linked to the extracellular portion of an IL-15RA polypeptide, or a fragment thereof, by a linker to form an IL-15/IL-15RA fusion; and~~

~~ii. a second exogenous stimulatory polypeptide, wherein the second exogenous stimulatory polypeptide comprises IL-12, or a fragment thereof,~~

~~wherein the cell is produced by a process comprising:~~

~~i. introducing a first exogenous nucleic acid encoding the first exogenous stimulatory polypeptide into a nucleated erythroid cell;~~

~~ii. introducing a second exogenous nucleic acid encoding the second exogenous stimulatory polypeptide into the nucleated erythroid cell; and~~

~~iii. culturing the nucleated erythroid cell under conditions suitable for enucleation of the nucleated erythroid cell and for production of the first exogenous stimulatory polypeptide and the second exogenous stimulatory polypeptide.~~

~~146. An engineered enucleated erythroid cell, comprising:~~

~~i. a first exogenous stimulatory polypeptide, wherein the first exogenous stimulatory polypeptide comprises 4-1BBL , or a fragment thereof; and~~

~~ii. a second exogenous stimulatory polypeptide, wherein the second exogenous stimulatory polypeptide comprises IL-12, or a fragment thereof,~~

~~wherein the cell is produced by a process comprising:~~

~~i. introducing a first exogenous nucleic acid encoding the first exogenous stimulatory polypeptide into a nucleated erythroid cell;~~

~~ii. introducing a second exogenous nucleic acid encoding the second exogenous stimulatory polypeptide into the nucleated erythroid cell; and~~

~~iii. culturing the nucleated erythroid cell under conditions suitable for enucleation of the nucleated erythroid cell and for production of the first exogenous stimulatory polypeptide and the second exogenous stimulatory polypeptide.~~

147. The engineered enucleated erythroid cell of any one of claims 143-146, wherein the introducing step comprises transfecting the nucleated cell with a single lentiviral vector comprising both the first exogenous nucleic acid and the second exogenous nucleic acid.

148. The engineered enucleated erythroid cell of claims 143-146, wherein the introducing step comprises transfecting the nucleated erythroid cell with both a first lentiviral vector comprising the first exogenous nucleic acid and a second lentiviral vector comprising the second exogenous nucleic acid.

149. An engineered enucleated erythroid cell, comprising at least one exogenous stimulatory polypeptide selected from the group consisting of: IL-15/IL-15RA fusion, MHC class I chain-related protein A (MICA), MHC class I chain-related protein B (MICB) and insulin-like growth factor 1 (IGF-1), at the surface of the engineered enucleated cell, produced by a process comprising:

introducing an exogenous nucleic acid encoding the at least one exogenous stimulatory polypeptide into a nucleated erythroid cell; and

culturing the nucleated erythroid cell under conditions suitable for enucleation of the nucleated erythroid cell and for production of the at least one exogenous stimulatory polypeptide.

150. The method of any one of claims 142-149, wherein the exogenous nucleic acid comprises DNA.

151. The method of any one of claims 142-149, wherein the exogenous nucleic acid comprises RNA.

152. The method of any one of claims 142-149, wherein the introducing step comprises viral transduction.

153. The method of any one of claims 142-149, wherein the introducing step comprises electroporation.

~~154. The method of any one of claims 142-149, wherein the introducing step comprises utilizing one or more of: liposome mediated transfer, adenovirus, adeno-associated virus, herpes virus, a retroviral based vector, lipofection, and a lentiviral vector.~~

~~155. The method of any one of claims 142-149, wherein the introducing step comprises introducing the exogenous nucleic acid by transfection of a lentiviral vector.~~

~~156. The method of claim 154 or 155, wherein the lentiviral vector comprises a promoter selected from the group consisting of: beta-globin promoter, murine stem cell virus (MSCV) promoter, Gibbon ape leukemia virus (GALV) promoter, human elongation factor 1alpha (EF1alpha) promoter, CAG CMV immediate early enhancer and the chicken beta-actin (CAG), and human phosphoglycerate kinase 1 (PGK) promoter.~~

1. An enucleated erythroid cell engineered to stimulate an immune killer cell, wherein the enucleated erythroid cell comprises:

a first exogenous stimulatory polypeptide, wherein the first exogenous stimulatory polypeptide is a fusion polypeptide comprising a heterologous transmembrane domain from a polypeptide selected from the group consisting of: glycophorin A, glycophorin B, cluster of differentiation (CD) 147, CD44, CD58, intercellular adhesion molecule 4, basal cell adhesion molecule, complement receptor 1, CD99, erythroblast membrane-associated protein (ERMAP), junctional adhesion molecule A, neuroplastin, amphoterin-induced protein 2, Down Syndrome cell adhesion molecule-like 1, small integral membrane protein 1, transferrin receptor, Fas ligand, and Kell; and

a second exogenous stimulatory polypeptide,

wherein the first and second exogenous stimulatory polypeptides are sufficient to stimulate the immune killer cell.

2. The enucleated erythroid cell of claim 1, wherein the immune killer cell is a natural killer (NK) cell or a $CD8^+$ T cell.

3. The enucleated erythroid cell of claim 1, wherein the enucleated erythroid cell further comprises a third exogenous stimulatory polypeptide.

4. The enucleated erythroid cell of claim 1, wherein one or both of the first and second exogenous stimulatory polypeptides comprises a polypeptide selected from the group consisting of: interleukin (IL)-1 (IL-1), IL-2, IL-12, IL-15, IL-15/IL-15 receptor alpha (IL-15/IL-15RA) fusion, IL-18, IL-21, interferon alpha (IFNα), 4-1BBL, poliovirus receptor (PVR), CD48, human leukocyte antigen (HLA)-A, HLA-C, HLA-G, heparan sulfate, HLA-E, immunoglobulin G (IgG), a MHC class I chain-related (MIC) protein, B7-H6, NK cell activating receptor 44 ligand, Nectin2, NK-T-B antigen, activation-induced C-type lectin (AICL), and insulin-like growth factor 1.

5. The enucleated erythroid cell of claim 4, wherein the MIC protein is selected from the group consisting of: MICA, MICB, and UL16-binding proteins (ULBP).

6. The enucleated erythroid cell of claim 1, wherein the first exogenous stimulatory polypeptide comprises IL-15/IL-15RA fusion and the second exogenous stimulatory polypeptide comprises a polypeptide selected from the group consisting of: IL-1, IL-2, IL-12, IL-18, IL-21, 4-1BBL, IFNα, MICA, MICB, PVR, and CD48.

7. The enucleated erythroid cell of claim 1, wherein the first exogenous stimulatory polypeptide and the second exogenous stimulatory polypeptide are selected from the group consisting of: IL-18 and IL-12, IL-18 and IL-21, IL-12 and 4-1BBL, IL-12 and IL-15/IL-15RA fusion, and 4-1BBL and IL-15/IL-15RA fusion.

8. The enucleated erythroid cell of claim 1, wherein the second exogenous stimulatory polypeptide is a fusion polypeptide.

9. The enucleated erythroid cell of claim 3, wherein the second and the third exogenous stimulatory polypeptides are present as fusion polypeptides.

10. An engineered enucleated erythroid cell comprising a first exogenous stimulatory polypeptide, wherein the first exogenous stimulatory polypeptide is a fusion polypeptide comprising (i) an interleukin-15 (IL-15) polypeptide, or a fragment thereof, (ii) an extracellular portion of an interleukin-15 receptor alpha (IL-15RA) polypeptide, or a fragment thereof, and (iii) a heterologous transmembrane domain from a polypeptide selected from the group consisting of: glycophorin A, glycophorin B, CD147, CD44, CD58, intercellular adhesion molecule 4, basal cell adhesion molecule, complement receptor 1, CD99, ERMAP, junctional adhesion molecule A, neuroplastin, amphoterin-induced protein 2, Down

Syndrome cell adhesion molecule-like 1, small integral membrane protein 1, transferrin receptor, Fas ligand, and Kell.

11. The engineered enucleated erythroid cell of claim 10, wherein the IL-15 polypeptide and the extracellular portion of an IL-15RA polypeptide are present as a complex.

12. The engineered enucleated erythroid cell of claim 10, wherein the fusion polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 1.

13. The engineered enucleated erythroid cell of claim 10, wherein the fusion polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 29 or SEQ ID NO: 37.

14. The engineered enucleated erythroid cell of claim 10, wherein the fusion polypeptide comprises an IL-15 polypeptide, or a fragment thereof, and an IL-15 receptor alpha sushi domain.

15. The engineered enucleated erythroid cell of claim 14, wherein the fusion polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 2 or SEQ ID NO: 31.

16. The engineered enucleated erythroid cell of claim 10, further comprising a second exogenous stimulatory polypeptide, wherein the second exogenous stimulatory polypeptide comprises 4-1BBL.

17. The engineered enucleated erythroid cell of claim 16, wherein the second exogenous stimulatory polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 41 or SEQ ID NO: 43.

18. The engineered enucleated erythroid cell of claim 10, further comprising a second exogenous stimulatory polypeptide, wherein the second exogenous stimulatory polypeptide comprises IL-12.

19. The engineered enucleated erythroid cell of claim 18, wherein the second exogenous stimulatory polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 37 or SEQ ID NO: 55.

20. An engineered enucleated erythroid cell comprising at least a first exogenous stimulatory polypeptide comprising a fusion polypeptide comprising (i) a polypeptide selected from the group consisting of: MICA, MICB, and IGF-1, and (ii) a heterologous transmembrane domain from a polypeptide selected from the group consisting of: glycophorin A, glycophorin B, CD147, CD44, CD58, intercellular adhesion molecule 4, basal cell adhesion molecule, complement receptor 1, CD99, ERMAP, junctional adhesion molecule A, neuroplastin, amphoterin-induced protein 2, Down Syndrome cell adhesion molecule-like 1, small integral membrane protein 1, transferrin receptor, Fas ligand, and Kell.

21. A method of stimulating an immune killer cell, comprising contacting the immune killer cell with the enucleated erythroid cell of claim 1 or the engineered enucleated erythroid cell of claim 10, in an amount effective to stimulate the immune killer cell.

22. The method of claim 21, wherein the enucleated erythroid cell or the engineered enucleated erythroid cell is to be administered to a subject in need of immune killer cell activation.

23. The method of claim 22, wherein the subject has cancer or an infectious disease.

24. The method of claim 23, wherein the cancer is selected from the group consisting of: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), anal cancer, bile duct cancer, bladder cancer, bone cancer, bowel cancer, brain tumor, breast cancer, cancer of unknown primary, cancer spread to bone, cancer spread to brain, cancer spread to liver, cancer spread to lung, carcinoid, cervical cancer, choriocarcinoma, chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), colon cancer, colorectal cancer, endometrial cancer, eye cancer, gallbladder cancer, gastric cancer, gestational trophoblastic tumor (GTT), hairy cell leukemia, head and neck cancer, Hodgkin's lymphoma, kidney cancer, laryngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma skin cancer, mesothelioma, men's cancer, molar pregnancy, mouth and oropharyngeal cancer, myeloma, nasal and sinus cancers, nasopharyngeal cancer, non-Hodgkin's lymphoma (NHL), oesophageal cancer, ovarian cancer, pancreatic cancer, penile cancer, prostate cancer, rare cancers, rectal cancer, salivary gland cancer, secondary cancers, skin cancer (non-melanoma), soft tissue sarcoma, stomach cancer, testicular cancer, thyroid cancer, unknown primary cancer, uterine cancer, vaginal cancer, and vulvar cancer.

25. Use of an enucleated erythroid cell of claim 1 or an engineered enucleated erythroid cell of claim 10, in the manufacture of a medicament for the treatment of a cancer in a subject, wherein the

enucleated erythroid cell or the engineered enucleated erythroid cell is to be administered in an amount effective to treat the cancer in the subject.

26. The use of claim 25, wherein the cancer is selected from the group consisting of: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), anal cancer, bile duct cancer, bladder cancer, bone cancer, bowel cancer, brain tumor, breast cancer, cancer of unknown primary, cancer spread to bone, cancer spread to brain, cancer spread to liver, cancer spread to lung, carcinoid, cervical cancer, choriocarcinoma, chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), colon cancer, colorectal cancer, endometrial cancer, eye cancer, gallbladder cancer, gastric cancer, gestational trophoblastic tumor (GTT), hairy cell leukemia, head and neck cancer, Hodgkin's lymphoma, kidney cancer, laryngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma skin cancer, mesothelioma, men's cancer, molar pregnancy, mouth and oropharyngeal cancer, myeloma, nasal and sinus cancers, nasopharyngeal cancer, non-Hodgkin's lymphoma (NHL), oesophageal cancer, ovarian cancer, pancreatic cancer, penile cancer, prostate cancer, rare cancers, rectal cancer, salivary gland cancer, secondary cancers, skin cancer (non-melanoma), soft tissue sarcoma, stomach cancer, testicular cancer, thyroid cancer, unknown primary cancer, uterine cancer, vaginal cancer, and vulvar cancer.

27. Use of an enucleated erythroid cell of claim 1 or an engineered enucleated erythroid cell of claim 10, in the manufacture of a medicament for the treatment of an infectious disease in a subject, wherein the enucleated erythroid cell or the engineered enucleated erythroid cell is to be administered in an amount effective to treat the infectious disease in the subject.

28. A method of making the enucleated erythroid cell of claim 1, the method comprising: introducing at least one exogenous nucleic acid into a nucleated erythroid cell precursor, wherein the at least one exogenous nucleic acid encodes the first and second exogenous stimulatory polypeptides; and culturing the nucleated erythroid cell precursor under conditions suitable for enucleation of the nucleated erythroid cell precursor and for production of the first and second exogenous stimulatory polypeptides, thereby making the enucleated erythroid cell.

29. An engineered enucleated erythroid cell, comprising:

i. a first exogenous stimulatory polypeptide, wherein the first exogenous stimulatory polypeptide is a fusion polypeptide comprising an IL-15/IL-15RA fusion comprising an IL-15 polypeptide, or a fragment thereof, linked to the extracellular portion of an IL-15RA polypeptide, or a fragment thereof, by a linker; and

ii. a second exogenous stimulatory polypeptide, wherein the second exogenous stimulatory polypeptide comprises 4-1BBL, or a functional fragment thereof,

wherein one or both of the first exogenous stimulatory polypeptide and the second exogenous stimulatory polypeptide comprise a heterologous transmembrane domain from a polypeptide selected from the group consisting of: glycophorin A, glycophorin B, CD147, CD44, CD58, intercellular adhesion molecule 4, basal cell adhesion molecule, complement receptor 1, CD99, ERMAP, junctional adhesion molecule A, neuroplastin, amphoterin-induced protein 2, Down Syndrome cell adhesion molecule-like 1, small integral membrane protein 1, transferrin receptor, Fas ligand, and Kell.

30. An engineered enucleated erythroid cell, comprising:

i. a first exogenous stimulatory polypeptide, wherein the first exogenous stimulatory polypeptide comprises 4-1BBL, or a fragment thereof; and

ii. a second exogenous stimulatory polypeptide, wherein the second exogenous stimulatory polypeptide comprises IL-12, or a fragment thereof,

wherein one or both of the first exogenous stimulatory polypeptide and the second exogenous stimulatory polypeptide is a fusion polypeptide further comprising a heterologous transmembrane domain from a polypeptide selected from the group consisting of: glycophorin A, glycophorin B, CD147, CD44, CD58, intercellular adhesion molecule 4, basal cell adhesion molecule, complement receptor 1, CD99, ERMAP, junctional adhesion molecule A, neuroplastin, amphoterin-induced protein 2, Down Syndrome cell adhesion molecule-like 1, small integral membrane protein 1, transferrin receptor, Fas ligand, and Kell.

31. An enucleated erythroid cell of claim 1 or an engineered enucleated erythroid cell of claim 10, for use in therapy.