CLAIMS

1. An engineered cytokine signaling chain, encoding a transmembrane polypeptide when expressed in an immune cell, wherein the transmembrane polypeptide comprises an extracellular portion and an intracellular portion, operably connected by a transmembrane portion, wherein:

the intracellular portion comprises a variant intracellular domain (ICD) of interleukin-9 receptor (IL9R), comprising at least one of a first sequence alteration in a first region corresponding positions 174-230, or a second sequence alteration in a second region corresponding positions 142-151, of the sequence as set forth in SEQ ID NO: 1.

2. The engineered cytokine signaling chain of claim 1, wherein each of the first sequence alteration or the second sequence alteration comprises a truncation or a substitution; optionally wherein the first sequence alteration comprises a truncation at positions 174-230 of the sequence as set forth in SEQ ID NO: 1, and the second sequence alteration comprises a substitution in the second region at positions 142-151 of the sequence as set forth in SEQ ID NO: 1; optionally wherein the variant ICD of IL9R in the intracellular portion comprises a sequence as set forth in SEQ ID NO: 7.

3. The engineered cytokine signaling chain of claim 1 or 2, wherein the extracellular portion comprises an extracellular domain (ECD) of a cytokine receptor chain or a functional variant thereof, optionally wherein:

i) the cytokine receptor chain is IL2Rβ, and the ECD of the cytokine receptor chain or the functional variant thereof in the extracellular portion comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 3;

ii) the cytokine receptor chain is IL4Rα, and the ECD of the cytokine receptor chain or the functional variant thereof in the extracellular portion comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 33;

iii) the cytokine receptor chain is IL7Rα, and the ECD of the cytokine receptor chain or the functional variant thereof in the extracellular portion comprises a sequence having at

least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 5;

iv) the cytokine receptor chain is IL9R, and the ECD of the cytokine receptor chain or the functional variant thereof in the extracellular portion comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 36; and/or

v) the cytokine receptor chain is IL21R, and the ECD of the cytokine receptor chain or the functional variant thereof in the extracellular portion comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 39.

4. The engineered cytokine signaling chain of claim 3, wherein:

the transmembrane portion comprises a transmembrane domain from the cytokine receptor chain; and/or

the transmembrane portion comprises a transmembrane domain from a polypeptide other than the cytokine receptor chain; optionally wherein: one of the following is met:

the cytokine receptor chain is IL2Rβ, and the transmembrane domain of the transmembrane portion comprises a sequence as set forth in SEQ ID NO: 19;

the cytokine receptor chain is IL4Rα, and the transmembrane domain of the transmembrane portion comprises a sequence as set forth in SEQ ID NO: 34;

the cytokine receptor chain is IL7Rα, and the transmembrane domain of the transmembrane portion comprises a sequence as set forth in SEQ ID NO: 24;

the cytokine receptor chain is IL9R, and the transmembrane domain of the transmembrane portion comprises a sequence as set forth in SEQ ID NO: 37; or

the cytokine receptor chain is IL21R, and the transmembrane domain of the transmembrane portion comprises a sequence as set forth in SEQ ID NO: 40.

5. The engineered cytokine signaling chain of claim 3 or 4, wherein the extracellular portion further comprises a cytokine ligand domain (CLD) over an N-terminus of the ECD of the cytokine receptor chain or the functional variant thereof, wherein the CLD is functionally compatible to the ECD of the cytokine receptor chain or the functional variant thereof, optionally wherein:

the cytokine receptor chain is IL2Rβ, and the CLD comprises IL15 or a functional variant thereof, wherein the CLD comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 9;

the cytokine receptor chain is IL2Rβ, and the CLD comprises IL2 or a functional variant thereof, wherein the CLD comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 44;

the cytokine receptor chain is IL4Rα, and the CLD comprises IL4 or a functional variant thereof, wherein the CLD comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 49;

the cytokine receptor chain is IL7Rα, and the CLD comprises IL7 or a functional variant thereof, wherein the CLD comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 13;

the cytokine receptor chain is IL9R, and the CLD comprises IL9 or a functional variant thereof, wherein the CLD comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 46; or

the cytokine receptor chain is IL21R, and the CLD comprises IL21 or a functional variant thereof, wherein the CLD comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 47.

6. The engineered cytokine signaling chain of claim 5, wherein:

i) the CLD is connected to the N-terminus of the ECD of the cytokine receptor chain or the functional variant thereof via a flexible linker;

ii) when the engineered cytokine signaling chain is expressed in an immune cell, the immune cell exhibits increased STAT3 signaling in the absence of IL-2 stimulation compared to when the engineered cytokine signaling chain is not expressed in the immune cell;

iii) when the engineered cytokine signaling chain is expressed in an immune cell, the immune cell displays increased proliferation capability when cultured *in vitro* in the absence of IL-2, compared to when the engineered cytokine signaling chain is not expressed in the immune cell;

iv) when the engineered cytokine signaling chain is expressed in an immune cell, the immune cell displays increased viability when cultured in vitro in the absence of IL-2, compared to when the engineered cytokine signaling chain is not expressed in the immune cell; and/or

v) when the engineered cytokine signaling chain is expressed in an immune cell, the immune cell exhibits increased cytotoxicity against target cells of the immune cell when co-cultured with the target cells in the absence of IL-2 stimulation, compared to when the engineered cytokine signaling chain is not expressed in the immune cell.

7. The engineered cytokine signaling chain of claim 6, wherein one of the following is met:

the cytokine receptor chain is IL2Rβ, and the engineered cytokine signaling chain comprises a sequence as set forth in SEQ ID NO: 79 or SEQ ID NO: 80;

the cytokine receptor chain is IL4Rα, and the engineered cytokine signaling chain comprises a sequence as set forth in SEQ ID NO: 81;

the cytokine receptor chain is IL7Rα, and the engineered cytokine signaling chain comprises a sequence as set forth in SEQ ID NO: 82;

the cytokine receptor chain is IL9R, and the engineered cytokine signaling chain comprises a sequence as set forth in SEQ ID NO: 83; or

the cytokine receptor chain is IL21R, and the engineered cytokine signaling chain comprises a sequence as set forth in SEQ ID NO: 84.

8. The engineered cytokine signaling chain of claim 3 or 4, wherein the extracellular portion does not comprise a cytokine ligand domain (CLD) that is functionally compatible to the ECD of the cytokine receptor chain or the functional variant thereof; optionally wherein one of the following is met:

the cytokine receptor chain is IL2Rβ, and the engineered cytokine signaling chain comprises a sequence as set forth in SEQ ID NO: 8;

the cytokine receptor chain is IL4Rα, and the engineered cytokine signaling chain comprises a sequence as set forth in SEQ ID NO: 35;

the cytokine receptor chain is IL7Rα, and the engineered cytokine signaling chain comprises a sequence as set forth in SEQ ID NO: 12;

the cytokine receptor chain is IL9R, and the engineered cytokine signaling chain comprises a sequence as set forth in SEQ ID NO: 38; or

the cytokine receptor chain is IL21R, and the engineered cytokine signaling chain comprises a sequence as set forth in SEQ ID NO: 41.

9. The engineered cytokine signaling chain of claim 8, wherein:

the engineered cytokine ligand chain encodes a secreted cytokine ligand when expressed in the immune cell; or

the engineered cytokine ligand chain encodes a membrane-bound cytokine ligand when expressed in the immune cell.

10. A synthetic cytokine signaling system for expression in an immune cell, comprising an engineered cytokine signaling chain according to any one of claims 1-9.

11. The synthetic cytokine signaling system of claim 10, wherein:

i) the synthetic cytokine signaling system comprises an engineered cytokine signaling chain according to claim 8 or 9 , and further comprising an engineered cytokine ligand chain, wherein:

the extracellular portion of the engineered cytokine signaling chain comprises an extracellular domain (ECD) of a cytokine receptor chain or a functional variant thereof, wherein the cytokine receptor chain is selected from a group consisting of IL2Rβ, IL4Rα, IL7Rα, IL9R, and IL21R; and

when the engineered cytokine signaling chain and the engineered cytokine ligand chain are co-expressed in the immune cell, the engineered cytokine ligand chain is capable of activating the engineered cytokine signaling chain; or

the synthetic cytokine signaling system consists of an engineered cytokine signaling chain according to claim 5 or 5.

12. The synthetic cytokine signaling system of claim 11, wherein the engineered cytokine ligand chain encodes a secreted cytokine ligand; optionally wherein:

i) the extracellular portion of the engineered cytokine signaling chain comprises the extracellular domain (ECD) of IL2Rβ or a functional variant thereof, wherein the engineered cytokine ligand chain encodes at least one of:

a secreted IL2, comprising a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 44; or

a secreted IL15, comprising a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 9; or

ii) the extracellular portion of the engineered cytokine signaling chain comprises the extracellular domain (ECD) of IL4Rα or a functional variant thereof, wherein the engineered cytokine ligand chain encodes a secreted IL4, comprising a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 49;

iii) the extracellular portion of the engineered cytokine signaling chain comprises the extracellular domain (ECD) of IL7Rα or a functional variant thereof, wherein the engineered cytokine ligand chain encodes a secreted IL7, comprising a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 13;

iv) the extracellular portion of the engineered cytokine signaling chain comprises the extracellular domain (ECD) of IL9R or a functional variant thereof, wherein the engineered cytokine ligand chain encodes a secreted IL9, comprising a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 46; or

v) the extracellular portion of the engineered cytokine signaling chain comprises the extracellular domain (ECD) of IL21R or a functional variant thereof, wherein the engineered cytokine ligand chain encodes a secreted IL21, comprising a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 47.

13. The synthetic cytokine signaling system of claim 11, wherein the engineered cytokine ligand chain encodes a membrane-bound cytokine ligand, comprising a ligand portion operably connected to a membrane-bound portion, wherein the ligand portion is functionally compatible to the ECD of the cytokine receptor chain or the functional variant thereof in the extracellular portion of the engineered cytokine signaling chain; optionally wherein:

i) the extracellular portion of the engineered cytokine signaling chain comprises the extracellular domain (ECD) of IL2Rβ or a functional variant thereof, wherein the engineered cytokine ligand chain encodes at least one of:

a membrane-bound IL2, wherein the ligand portion thereof comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 44; or

a membrane-bound IL15, wherein the ligand portion thereof comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 9;

ii) the extracellular portion of the engineered cytokine signaling chain comprises the extracellular domain (ECD) of IL4Rα or a functional variant thereof, wherein the engineered cytokine ligand chain encodes a membrane-bound IL4, wherein the ligand portion thereof comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 49;

iii) the extracellular portion of the engineered cytokine signaling chain comprises the extracellular domain (ECD) of IL7Rα or a functional variant thereof, wherein the engineered cytokine ligand chain encodes a membrane-bound IL7, wherein the ligand portion thereof comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 13;

iv) the extracellular portion of the engineered cytokine signaling chain comprises the extracellular domain (ECD) of IL9R or a functional variant thereof, wherein the engineered cytokine ligand chain encodes a membrane-bound IL9, wherein the ligand portion thereof comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 46; or

v) the extracellular portion of the engineered cytokine signaling chain comprises the extracellular domain (ECD) of IL21R or a functional variant thereof, wherein the engineered cytokine ligand chain encodes a membrane-bound IL21, wherein the ligand portion thereof comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 47.

14. The synthetic cytokine signaling system of claim 13, wherein the membrane-bound portion of the engineered cytokine ligand chain comprises a fragment crystallizable region (Fc) or a functional variant thereof;

optionally wherein:

i) the Fc comprises a IgG4Fc, wherein the membrane-bound portion comprises a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 14;

ii) the membrane-bound portion and the ligand portion is operably connected via a hinge, optionally wherein the hinge comprises a sequence as set forth in SEQ ID NO: 21;

iii) in the membrane-bound portion, the Fc or a functional variant thereof is operably connected to a transmembrane region, optionally wherein the transmembrane region comprises a sequence as set forth in SEQ ID NO: 22; and/or

iii) the engineered cytokine ligand chain comprises a sequence as set forth in any one of SEQ ID NO: 15 and 54-58.

15. The synthetic cytokine signaling system of claim 13, wherein the engineered cytokine ligand chain encodes a membrane-bound IL15, wherein the membrane-bound portion of the membrane-bound IL15 comprises IL15Rα or a functional variant thereof, comprising a sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% identity to the sequence as set forth in SEQ ID NO: 10; optionally wherein:

i) the membrane-bound portion comprises the sequence as set forth in SEQ ID NO: 10;

ii) the membrane-bound portion and the ligand portion is operably connected via a linker, optionally wherein the linker comprises a sequence as set forth in SEQ ID NO: 16; and/or

iii) the engineered cytokine ligand chain comprises a sequence as set forth in SEQ ID NO: 11.

16. A vector kit, comprising at least one vector, wherein when the at least one vector is introduced in an immune cell, the immune cell expresses the synthetic cytokine signaling system according to any one of claims 10-15; optionally wherein: i) the synthetic cytokine signaling system comprises an engineered cytokine signaling chain and an engineered cytokine ligand chain, wherein the vector kit consists of one vector, wherein the one vector comprises two nucleotide sequences respectively encoding the engineered cytokine signaling chain and the engineered cytokine ligand chain in the synthetic cytokine signaling system, optionally wherein the two nucleotide sequences are in a common open reading frame (ORF), and the one vector comprises a separator sequence separating the two nucleotide sequences, wherein the separator sequence comprises F2A or P2A, wherein the F2A comprises a sequence as set forth in SEQ ID NO: 42, and the P2A comprises a sequence as set forth in SEQ ID NO: 43;

ii) the two nucleotide sequences are in two different open reading frames (ORFs);

iii) the synthetic cytokine signaling system comprises an engineered cytokine signaling chain and an engineered cytokine ligand chain, wherein the vector kit comprises a first vector and a second vector, wherein:

the first vector comprises a first nucleotide sequence encoding the engineered cytokine signaling chain; and

the second vector comprises a second nucleotide sequence encoding the engineered cytokine ligand chain; and/or

iv) the synthetic cytokine signaling system consists of an engineered cytokine signaling chain, wherein the vector kit consists of one single vector encoding the engineered cytokine signaling chain.

17. A method for obtaining an engineered immune cell, comprising introducing into an immune cell a vector kit according to claim 16.

18. An immune cell, comprising the synthetic cytokine signaling system according to any one of claims 10-15; optionally wherein:

i) when cultured in *vitro* in the absence of IL-2, the immune cell displays at least one of increased proliferation capability, increased viability, or increased STAT signaling, compared to an immune cell that does not comprise the synthetic cytokine signaling system;

ii) when co-cultured with target cells of the immune cell in the absence of IL-2 stimulation, the immune cell displays at least one of increased interferon gamma (IFNγ) secretion or increased cytotoxicity against the target cells, compared to an immune cell that does not comprise the synthetic cytokine signaling system;

iii) when a population of the immune cell are transferred into a subject bearing a tumor that the immune cell specifically targets, the population of the immune cell exhibit more preferred migration to tumor tissues and/or lymphoid tissues, and/or displays increased tumor control ability, compared to an immune cell that does not comprise the synthetic cytokine signaling system; and/or

iv) the immune cell further expresses a targeting polypeptide, wherein the targeting polypeptide comprises at least one of a chimeric antigen receptor (CAR), a T cell receptor (TCR), or an Aspire-T cell receptor; and/orv) wherein the immune cell is any of a T cell, a γδ T cell, a natural killer (NK) cell, a natural killer T (NKT) cell, or a tumor-infiltrating lymphocyte (TIL), or an engineered immune cell derived therefrom.

19. A method, comprising:

obtaining an engineered immune cell based on an immune cell, such that the engineered immune cell expresses the synthetic cytokine signaling system according to any one of claims 10-15; and

culturing the engineered immune cell in a medium containing none or a reduced level of a cytokine supplement *in vitro*, wherein the cytokine supplement comprises at least one of IL-2, IL-4, IL-7, IL-9, IL-15, or IL-21, optionally wherein the cytokine supplement consists of IL-2.

20. Use of a population of engineered immune cells in the manufacture of a medicament for treating a disease in a subject, wherein each of the population of the engineered immune cells expresses the synthetic cytokine signaling system according to any one of claims 10-15; optionally wherein each of the engineered immune cells has been obtained from an immune cell; and cultured in a medium containing none or a reduced level of a cytokine *supplement in vitro* to thereby obtain the population of the engineered immune cells; optionally wherein the engineered immune cell further expresses a targeting polypeptide, wherein the targeting polypeptide comprises at least one of a chimeric antigen receptor (CAR), a T cell receptor (TCR), or an Aspire-T cell receptor; optionally wherein the immune cell is any of a T cell, a γδ T cell, a natural killer (NK) cell, a natural killer T (NKT) cell, or a tumor-infiltrating lymphocyte (TIL), or an engineered immune cell derived therefrom; and optionally wherein the disease is a cancer, an autoimmune disease, or a viral infection.