Claims

1. A method of making an engineered cell, which has reduced or eliminated surface expression of MHC class II protein relative to an unmodified cell, comprising contacting a cell with a composition comprising:

a. a CIITA guide RNA comprising

i) a guide sequence selected from SEQ ID NOs: 1-117;

ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-117;

iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-117;

iv) a sequence that comprises 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Table 2;

v) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (iv); or

vi) a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (v); and

b. optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

2. A method of reducing or eliminating surface expression of MHC class II protein in an engineered cell relative to an unmodified cell, comprising contacting a cell with a composition comprising:

a. a CIITA guide RNA comprising

i) a guide sequence selected from SEQ ID NOs: 1-117;

ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 1-117;

iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NOs: 1-117;

iv) a sequence that comprises 10 contiguous nucleotides ±10 nucleotides of a genomic coordinate listed in Table 2;

v) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence from (iv); or

vi) a guide sequence that is at least 95%, 90%, or 85% identical to a sequence selected from (v); and

b. optionally an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent.

3. A method of making an engineered cell, which has reduced or eliminated surface expression of MHC class II protein and HLA-A protein relative to an unmodified cell, comprising:

a. contacting the cell with a CIITA guide RNA, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 1-117;

b. contacting the cell with an HLA-A guide RNA, wherein the HLA-A guide RNA comprises a guide sequence selected from any one of SEQ ID NOs: 2001-2095; and

c. optionally contacting the cell with an RNA-guided DNA binding agent or nucleic acid encoding an RNA-guided DNA binding agent;

thereby reducing or eliminating the surface expression of MHC class II protein and HLA-A protein in the cell relative to an unmodified cell.

4. The method of any one of claims 1-3, wherein the CIITA guide RNA comprises

i) a guide sequence selected from SEQ ID NOs: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115;

ii) at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NO: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115; or

iii) a guide sequence at least 95%, 90%, or 85% identical to a sequence selected from SEQ ID NO: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115.

5. The method of any one of claims 1-4, wherein the CIITA guide RNA comprises a sequence selected from SEQ ID NOs: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115.

6. The method of any one of claims 1-5, further comprising reducing or eliminating the surface expression of MHC class I protein in the cell relative to an unmodified cell; and/or further comprising reducing or eliminating the surface expression of B2M protein in the cell relative to an unmodified cell; and/or further comprising reducing or eliminating the surface expression of HLA-A protein in the cell relative to an unmodified cell; and/or further comprising reducing or eliminating the surface expression of a TCR protein in the cell relative to an unmodified cell; and/or further comprising contacting the cell with an exogenous nucleic acid, and optionally wherein the exogenous nucleic acid encodes a targeting receptor or a polypeptide that is secreted

by the cell; and/or further comprising contacting the cell with a DNA-dependent protein kinase inhibitor (DNAPKi), and optionally wherein the DNAPKi is Compound 1.

7. An engineered cell, which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10902662-10923285.

8. The engineered cell of claim 7, wherein the genetic modification comprises at least 5, 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates chr16:10902662-10923285; and/or wherein the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates chr16:10902662-10923285; and/or wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906542-10923285; and/or wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chr16:10906542-10908121.

9. The engineered cell of claim 7 or 8,

(i) wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285; and/or

(ii) wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642,

chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and chr16:10895302-10895322.

10. An engineered cell, which has reduced or eliminated surface expression of MHC class II relative to an unmodified cell, comprising a genetic modification in the CIITA gene, wherein the genetic modification comprises an indel, a C to T substitution, or an A to G substitution within the genomic coordinates chosen from: chr16:10902662-10902682, chr16:10902723-10902743, chr16:10902729-10902749, chr16:10903747-10903767, chr16:10903824-10903844, chr16:10903824-10903844, chr16:10903848-10903868, chr16:10904761-10904781, chr16:10904764-10904784, chr16:10904765-10904785, chr16:10904785-10904805, chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629, chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702, chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906757-10906777, chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-10906867, chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-10906933, chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-10907005, chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-10907139, chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-10907292, chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455, chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481, chr16:10907476-10907496, chr16:10907539-10907559, chr16:10907586-10907606, chr16:10907589-10907609, chr16:10907621-10907641, chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-10907944, chr16:10907928-10907948, chr16:10907932-10907952, chr16:10907935-10907955,

chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089, chr16:10908073-10908093, chr16:10908101-10908121, chr16:10909056-10909076, chr16:10909138-10909158, chr16:10910195-10910215, chr16:10910196-10910216, chr16:10915592-10915612, chr16:10915626-10915646, chr16:10916375-10916395, chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452, chr16:10918486-10918506, chr16:10918492-10918512, chr16:10918493-10918513, chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461, chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, and chr16:10923265-10923285.

11. The engineered cell of claim 10,

(i) wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, chr16:10907586-10907606, chr16:10907476-10907496, chr16:10906904-10906924, and chr16:10895302-10895322; and/or

(ii) wherein the genetic modification comprises at least one nucleotide of an exon within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285.

12. The engineered cell of claim 10 or 11, wherein the genetic modification comprises at least 5, 6, 7, 8, 9, or 10 contiguous nucleotides within the genomic coordinates; and/or wherein the genetic modification comprises at least one C to T substitution or at least one A to G substitution within the genomic coordinates.

13. The engineered cell of any one of claims 7-12,

(i) wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10902662-10902682, chr16:10902723-10902743, chr16:10902729-10902749, chr16:10903747-10903767, chr16:10903824-10903844, chr16:10903824-10903844, chr16:10903848-10903868, chr16:10904761-10904781, chr16:10904764-10904784, chr16:10904765-10904785, chr16:10904785-10904805, chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629, chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702, chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906757-10906777, chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-10906867, chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906853-10906873, chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-10906933, chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-10907005, chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-10907139, chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-10907292, chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455, chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481, chr16:10907476-10907496, chr16:10907539-10907559, chr16:10907586-10907606, chr16:10907589-10907609, chr16:10907621-10907641, chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-10907944, chr16:10907928-10907948, chr16:10907932-

10907952, chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089, chr16:10908073-10908093, chr16:10908101-10908121, chr16:10909056-10909076, chr16:10909138-10909158, chr16:10910195-10910215, chr16:10910196-10910216, chr16:10915592-10915612, chr16:10915626-10915646, chr16:10916375-10916395, chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452, chr16:10918486-10918506, chr16:10918492-10918512, chr16:10918493-10918513, chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461, chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, and chr16:10923265-10923285;

(ii) wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629, chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702, chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906757-10906777, chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-10906867, chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906853-10906873, chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-10906933, chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-10907005, chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-10907139, chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-10907292, chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455, chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481, chr16:10907476-10907496, chr16:10907539-10907559, chr16:10907586-10907606, chr16:10907589-10907609, chr16:10907621-10907641, chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840, chr16:10907870-10907890, chr16:10907886-10907906, chr16:10907924-10907944, chr16:10907928-

10907948, chr16:10907932-10907952, chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089, chr16:10908073-10908093, chr16:10908101-10908121, chr16:10909056-10909076, chr16:10909138-10909158, chr16:10910195-10910215, chr16:10910196-10910216, chr16:10915592-10915612, chr16:10915626-10915646, chr16:10916375-10916395, chr16:10916382-10916402, chr16:10916426-10916446, chr16:10916432-10916452, chr16:10918486-10918506, chr16:10918492-10918512, chr16:10918493-10918513, chr16:10922435-10922455, chr16:10922441-10922461, chr16:10922441-10922461, chr16:10922444-10922464, chr16:10922460-10922480, chr16:10923257-10923277, and chr16:10923265-10923285;

(iii) wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10906542-10906562, chr16:10906556-10906576, chr16:10906609-10906629, chr16:10906610-10906630, chr16:10906616-10906636, chr16:10906682-10906702, chr16:10906756-10906776, chr16:10906757-10906777, chr16:10906757-10906777, chr16:10906821-10906841, chr16:10906823-10906843, chr16:10906847-10906867, chr16:10906848-10906868, chr16:10906853-10906873, chr16:10906853-10906873, chr16:10906904-10906924, chr16:10906907-10906927, chr16:10906913-10906933, chr16:10906968-10906988, chr16:10906970-10906990, chr16:10906985-10907005, chr16:10907030-10907050, chr16:10907058-10907078, chr16:10907119-10907139, chr16:10907139-10907159, chr16:10907172-10907192, chr16:10907272-10907292, chr16:10907288-10907308, chr16:10907314-10907334, chr16:10907315-10907335, chr16:10907325-10907345, chr16:10907363-10907383, chr16:10907384-10907404, chr16:10907385-10907405, chr16:10907433-10907453, chr16:10907434-10907454, chr16:10907435-10907455, chr16:10907441-10907461, chr16:10907454-10907474, chr16:10907461-10907481, chr16:10907476-10907496, chr16:10907539-10907559, chr16:10907586-10907606, chr16:10907589-10907609, chr16:10907621-10907641, chr16:10907622-10907642, chr16:10907623-10907643, chr16:10907730-10907750, chr16:10907731-10907751, chr16:10907757-10907777, chr16:10907781-10907801, chr16:10907787-10907807, chr16:10907790-10907810, chr16:10907810-10907830, chr16:10907820-10907840, chr16:10907870-

10907890, chr16:10907886-10907906, chr16:10907924-10907944, chr16:10907928-10907948, chr16:10907932-10907952, chr16:10907935-10907955, chr16:10907978-10907998, chr16:10907979-10907999, chr16:10908069-10908089, chr16:10908073-10908093, and chr16:10908101-10908121;

(iv) wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10916432-10916452, chr16:10922444-10922464, chr16:10907924-10907944, chr16:10906985-10907005, chr16:10908073-10908093, chr16:10907433-10907453, chr16:10907979-10907999, chr16:10907139-10907159, chr16:10922435-10922455, chr16:10907384-10907404, chr16:10907434-10907454, chr16:10907119-10907139, chr16:10907539-10907559, chr16:10907810-10907830, chr16:10907315-10907335, chr16:10916426-10916446, chr16:10909138-10909158, chr16:10908101-10908121, chr16:10907790-10907810, chr16:10907787-10907807, chr16:10907454-10907474, chr16:10895702-10895722, chr16:10902729-10902749, chr16:10918492-10918512, chr16:10907932-10907952, chr16:10907623-10907643, chr16:10907461-10907481, chr16:10902723-10902743, chr16:10907622-10907642, chr16:10922441-10922461, chr16:10902662-10902682, chr16:10915626-10915646, chr16:10915592-10915612, chr16:10907385-10907405, chr16:10907030-10907050, chr16:10907935-10907955, chr16:10906853-10906873, chr16:10906757-10906777, chr16:10907730-10907750, and chr16:10895302-10895322; and/or

(v) wherein the MHC class II expression is reduced or eliminated by a gene editing system that binds to a CIITA genomic target sequence comprising at least 5 contiguous nucleotides within the genomic coordinates chosen from: chr16:10907539-10907559, chr16:10916426-10916446, chr16:10906907-10906927, chr16:10895702-10895722, chr16:10907757-10907777, chr16:10907623-10907643, chr16:10915626-10915646, chr16:10906756-10906776, chr16:10907476-10907496, chr16:10907385-10907405, and chr16:10923265-10923285.

14. The engineered cell of claim 13, wherein the CIITA genomic target sequence comprises at least 10 or at least 15 contiguous nucleotides within the genomic coordinates; and/or wherein the gene editing system comprises an RNA-guided DNA-binding agent, optionally wherein the RNA-guided DNA-binding agent comprises a Cas9 protein, such as an *S. pyogenes* Cas9.

15. The engineered cell of any one of claims 7-14, wherein the engineered cell further has reduced or eliminated surface expression of MHC class I, and optionally wherein the engineered cell comprises a genetic modification in the beta-2-microglobulin (B2M) gene, and optionally wherein the engineered cell comprises a genetic modification in an HLA-A gene.

16. The engineered cell of any one of claims 7-15, wherein the engineered cell comprises an exogenous nucleic acid encoding a targeting receptor that is expressed on the surface of the engineered cell, and optionally wherein the targeting receptor is a CAR, a T-cell receptor (TCR), or a WT1 TCR.

17. The engineered cell of any one of claims 7-16, wherein the engineered cell further comprises an exogenous nucleic acid encoding a polypeptide that is secreted by the engineered cell; and/or wherein the engineered cell is a T cell and further has reduced or eliminated expression of an endogenous T-cell receptor (TCR) protein relative to an unmodified cell, and optionally wherein the cell has reduced or eliminated expression of a TRAC protein or a TRBC protein relative to an unmodified cell.

18. A pharmaceutical composition comprising the engineered cell of any one of claims 7-17.

19. A population of cells comprising the engineered cell of any one of claims 7-17.

20. A pharmaceutical composition comprising a population of cells, wherein the population of cells comprises the engineered cell of any one of claims 7-17.

21. The population of cells of claim 19 or pharmaceutical composition of claim 18 or 20, wherein the population of cells is at least 65%, at least 70%, at least 80%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% MHC class II negative as measured by flow cytometry; and/or wherein the population of cells is at least 95%, at least 97%, at least 98%, or at least 99% endogenous TCR protein negative as measured by flow cytometry.

22. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-21, comprising an exogenous nucleic acid or contacting the cell with an exogenous nucleic acid, wherein the exogenous nucleic acid encodes an NK cell inhibitor molecule, and optionally wherein the NK cell inhibitor molecule binds to an inhibitory receptor on an NK cell, or optionally wherein the NK cell inhibitor molecule binds to NKG2A on an NK cell, or optionally wherein the NK cell inhibitor molecule is a non-classical MHC class I molecule, or optionally wherein the NK cell inhibitor molecule is HLA-E, or optionally wherein the NK cell inhibitor

molecule is a fusion protein, or optionally wherein the NK cell inhibitor molecule is a fusion protein comprising HLA-E and B2M.

23. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-22, comprising an exogenous nucleic acid encoding a polypeptide that is secreted by the cell or contacting the cell with said exogenous nucleic acid, wherein the secreted polypeptide is an antibody or antibody fragment, or wherein the secreted polypeptide is a full-length IgG antibody, a single chain antibody, or a neutralizing antibody, or wherein the secreted polypeptide is an enzyme, a cytokine, or a fusion protein, or wherein the secreted polypeptide comprises a soluble receptor.

24. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-23, comprising an exogenous nucleic acid encoding a targeting receptor or contacting the cell with an exogenous nucleic acid encoding a targeting receptor, wherein the targeting receptor is a T cell receptor (TCR), a genetically modified TCR, a WT1 TCR, or a CAR.

25. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-6 and 22-24, wherein the CIITA guide RNA, the RNA-guided DNA binding agent, and/or the exogenous nucleic acid is provided to the cell in a vector, optionally wherein the CIITA guide RNA and the RNA-guided DNA binding agent are provided in the same vector, and optionally wherein the vector is a viral vector or a non-viral vector, or optionally wherein the vector is a lentiviral vector or an AAV.

26. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-25, wherein a lipid nucleic acid assembly composition comprising a gene editing system component is provided to the cell, optionally wherein the lipid nucleic acid assembly composition is a lipid nanoparticle (LNP).

27. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-6 and 22-26, wherein a lipid nucleic acid assembly composition comprising the CIITA guide RNA and/or the exogenous nucleic acid, and optionally the RNA-guided DNA binding agent, is provided to the cell, optionally wherein the lipid nucleic acid assembly composition is a lipid nanoparticle (LNP).

28. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-6 and 22-27, wherein

(i) the CIITA guide RNA is a single guide RNA comprising any one of the sequences of SEQ ID NO: 335-426 and 1008 or a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any one of the sequences of SEQ ID NO: 335-426 and 1008;

(ii) the CIITA guide RNA comprises any one of the sequences of SEQ ID NOs: 32, 64, 67, 68, 74, 76, 84, 86, 90, 91, and 115; or

(iii) the CIITA guide RNA is a single guide RNA comprising any one of the sequences of SEQ ID NO: 341, 373, 376, 377, 383, 385, 393, 395, 399, 400, and 424, or a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any one of the sequences of SEQ ID NO: 341, 373, 376, 377, 383, 385, 393, 395, 399, 400, and 424.

29. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-6 and 22-28, wherein the CIITA guide RNA comprises at least one modification, wherein the at least one modification includes (i) a 2'-O-methyl (2'-O-Me) modified nucleotide, (ii) a phosphorothioate (PS) bond between nucleotides, (iii) a 2'-fluoro (2'-F) modified nucleotide, (iv) a modification at one or more of the first five nucleotides at the 5' end of the guide RNA, (v) a modification at one or more of the last five nucleotides at the 3' end of the guide RNA, (vi) a PS bond between the first four nucleotides of the guide RNA, (vii) a PS bond between the last four nucleotides of the guide RNA, (viii) a 2'-O-Me modified nucleotide at the first three nucleotides at the 5' end of the guide RNA, (ix) a 2'-O-Me modified nucleotide at the last three nucleotides at the 3' end of the guide RNA, or combinations of one or more of (i)-(ix).

30. An engineered cell or population of cells comprising a genetic modification that includes an indel, a C to T substitution, or an A to G substitution within the genomic region targeted by the CIITA guide RNA of any one of claims 1-6 and 22-29.

31. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-30, for use in expressing a TCR with specificity for a polypeptide expressed by cancer cells.

32. The engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-31, for use as an adoptive cell transfer (ACT) therapy to be administered to a subject.

33. Use of the engineered cell, population of cells, pharmaceutical composition, or method of any one of claims 1-32, in the manufacture of a medicament for treating a cancer, an infectious disease, or an autoimmune disease.

34. The engineered cell, population of cells, pharmaceutical composition, method, or use of any one of claims 1-33, wherein the genetic modification comprises:

(i) an indel;

(ii) a C to T substitution; and/or

(iii) an A to G substitution.

35. The engineered cell, population of cells, pharmaceutical composition, method, or use of any one of claims 1-34, wherein:

(i) the cell is homozygous for HLA-B and homozygous for HLA-C;

(ii) the cell further comprises a genetic modification in an HLA-A gene, wherein the cell is homozygous for HLA-B and homozygous for HLA-C, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from:

a. chr6:29942854 to chr6:29942913 and
b. chr6:29943518 to chr6: 29943619; and/or

(iii) the cell further comprises a genetic modification in an HLA-A gene, and wherein the genetic modification in the HLA-A gene comprises at least one nucleotide within the genomic coordinates chosen from:

a. chr6:29942864 to chr6: 29942903, and
b. chr6:29943528 to chr6:29943609.