SUMMARY

[0008] Provided herein are immune cells that have been engineered e.g. genetically engineered to mitigate rejection by a host or recipient into which or whom the cells have been introduced, methods of mitigating rejection and/or recognition by a host or recipient's immune system e.g. T cells and/or NK cells, compositions and populations comprising the engineered cells and methods of treating a cancer in a patient using the same. The immune cells of the instant disclosure are engineered to functionally express a reduced level, relative to corresponding cells that have not been so engineered, of one or both of NLRC5 and TAP2, two genes whose products participate in antigen presentation by cells, or of any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK. The engineered cells can be further engineered to augment resistance to rejection and/or to provide a therapeutic effect, e.g. the cells can be engineered to comprise or express an additional protein, e.g. an antigen binding protein such as a chimeric antigen receptor (CAR) and/or a T cell receptor, wherein the antigen binding protein or T cell receptor targets the engineered immune cell to tumor cells that express a cognate antigen and/or to other undesired e.g. disease state cells. The present disclosure thus provides a method of increasing persistence of allogeneic cells in a recipient, the method comprising engineering the cells to functionally express a reduced level of one or both of NLRC5 and TAP2, one or both of RFX5 and NLRC5, one or both of RFX5 and TAP2, or of any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK, relative to non-engineered cells.

[0009] In an example, the present disclosure provides an engineered immune cell that functionally expresses any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK at a reduced level. In some examples, the reduced level of expression, stated relative to the expression level in a corresponding but non-engineered immune cell, is 0%, for example when both chromosomal copies of a gene are knocked out, or 50% (i.e. 50% of the level in a non-engineered control immune cell), for example when one of the two chromosomal copies of a gene is knocked out and there is no compensatory increase in expression of the other chromosomal copy of that gene. In some examples, the cell expresses any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK at a level not greater than 90%, not greater than 75%, not greater than 50%, not greater than 25%, or not greater than 10% of the expression level in a non-engineered immune cell. In some examples, the level of expression of any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK in the engineered immune cell

is any value between 0% and 90% of the level in a control cell not correspondingly engineered with respect to the corresponding gene. In some examples, the expression level in the engineered cell is, for example, between 10% and 90%, between 25% and 90%, between 25% and 75%, between 10% and 50%, between 25% and 50%, between 50% and 90%, or between 50% and 75% of the level in a control cell. In some examples, a reduced level of expression other than 0% or 50% is obtained when, for example, only one chromosomal copy of a gene is knocked out and a compensatory mechanism causes an increase in the level of expression of the remaining chromosomal copy, or reduction in expression is achieved by a method other than gene knockout, such as known knockdown methods e.g. those that employ any of various RNA-based techniques (e.g. antisense RNA, miRNA, siRNA; *see, e.g.*, Lam *et al.*, Mol. Ther.-Nucleic Acids 4:e252 (2015), doi:10.1038/mtna.2015.23; Sridharan and Gogtay, Brit. J. Clin. Pharmacol. 82: 659-72 (2016)) In some examples, the engineered immune cell disclosed herein exhibits a reduced level of expression of an MHC class I protein or complex at the cell surface relative to a suitable control. In various examples, the cell is a T cell e.g. a human T cell. In some examples, the cell comprises a mutation in any one or more of the TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK loci or genes and/or a disruption in any one or more of the TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK loci or genes that causes a reduction in functional expression of the disrupted locus or gene. In an example, the mutation or disruption is introduced by any one or a combination of gene mutations or gene editing techniques, including but not limited to known homologous recombination techniques and techniques that employ any one or more of meganucleases, TALEN, zinc fingers, shRNA, Cas-CLOVER, and a CRISPR/Cas system (for example, Cas9, Cas12 and MAD7). In some examples, the cell is a non-human cell, e.g. a primate cell or a non-primate mammalian cell. In some examples, the cell is a human cell. In some examples, the mutation or disruption is produced by knocking in a nucleic acid e.g. a nucleic acid that encodes one or more proteins or gene products to be expressed in the cell. In some examples, the nucleic acid encodes either or both of membrane glycoprotein UL18 (*see, e.g.*, UniProtKB - P08560 (UL18_HCMVA), accessed at https://www.uniprot.org/uniprot/P08560) and ADR. In various examples, the ADR (alloimmune defense receptor) comprises the same components or domains as the ADRs disclosed in Feiyan Mo *et al.*, Nature Biotechnol. (2021) 39(1): 56–63. doi:10.1038/s41587-020-0601-5 ("Mo *et al.* 2021") and/or in U.S. Patent Publication No. 2021/0077530 (March

18, 2021) ("the '530 Publication"), the contents of both of which are incorporated herein in their entirety. In some examples, the ADR is a 4-1BB ADR, OX40 ADR, or a CD40L ADR such as that disclosed in the '530 Publication.

[0010] In various examples, the engineered immune cell further expresses an antigen binding protein, for example, the engineered immune cell comprises a nucleic acid that encodes an antigen binding protein. In an example, the antigen binding protein is a chimeric antigen receptor (CAR). In an example, a nucleic acid encoding the antigen binding protein e.g. the CAR is inserted into a disrupted TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP or RFXANK locus or is inserted into the locus, thereby disrupting it. In various examples, the antigen binding protein is a T cell receptor (TCR). In an example, a nucleic acid encoding the TCR is inserted into a disrupted TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP or RFXANK locus or is inserted into the locus, thereby disrupting it. In an example, the engineered immune cell further comprises one or more genomic modifications, e.g. a modification of an endogenous genetic locus, for example, of one or more of the following: an endogenous CD70 gene, an endogenous TCRa gene and an endogenous CD52 gene. In various examples, the one or more genomic modifications cause a reduction or absence of functional expression of the gene that contains the modification.

[0011] The engineered immune cell can be derived from cells from any of various sources. The engineered immune cell can be prepared or derived from cells e.g. stem cells or immune cells from a person other than the person to whom the engineered immune cells will be administered, e.g. a donor (e.g. a healthy volunteer) other than the recipient, or can be prepared or derived from cells e.g. stem cells or immune cells from the person to whom the engineered immune cells will be administered (the recipient), or can be derived from one or more induced pluripotent stem cells (iPSCs). In an example, the immune cell is an immune cell obtained from a healthy volunteer, is obtained from a patient, or is derived from an iPSC.

[0012] In another example, the present disclosure provides a method of making the engineered immune cell disclosed herein. In an example, the method comprises the use of any gene editing technology, such as TALEN, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system, and/or the use of any known gene knockdown methods e.g. those that employ any of various RNA-based techniques (e.g. shRNA, antisense RNA, miRNA, siRNA; *see, e.g.*, Lam *et al.*,

Mol. Ther.-Nucleic Acids 4:e252 (2015), doi:10.1038/mtna.2015.23; Sridharan and Gogtay, Brit. J. Clin. Pharmacol. 82: 659-72 (2016)) to reduce functional expression of one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK. In an example, the method comprises or further comprises the introduction into the engineered immune cell of a nucleic acid encoding an antigen binding protein, e.g. a CAR or TCR. In an example, the method comprises or further comprises introducing into the genome of the engineered immune cell one or more genomic modifications of one or more of an endogenous TCRa gene and an endogenous CD52 gene. In an example, the one or more genomic modifications disrupts and/or prevents, wholly or partly, the functional expression of one or more of an endogenous TCRa gene and an endogenous CD52 gene.

[0013] In various examples of the present disclosure, the functional expression level of any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK is measured by determining the surface expression level of one or more HLA proteins, such as an HLA-A or HLA-B protein, or of beta2 microglobulin (B2M), or of both one or more HLA proteins and B2M on the surface of the engineered immune cell, or is measured by flow cytometry. In some examples, expression of any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK in engineered immune cells of the present disclosure is assayed by measuring the degree to which the engineered immune cells survive in the presence of effector cells e.g. T cells, in comparison to the degree to which not correspondingly engineered, but otherwise comparable e.g. identical, immune cells survive under the same conditions.

[0014] In some examples, an immune cell that has been engineered to functionally express a reduced level, relative to corresponding cells that have not been so engineered, of one or both of NLRC5 and TAP2, is further engineered to express one or more proteins selected from the group consisting of HLA-E, HLA-E single-chain trimer, HLA-G, HLA-G single-chain trimer, UL18, UL18 single-chain trimer, HLA-A2, HLA-A2 single-chain trimer, human cytomegalovirus (HCMV) US11 and/or to express a reduced level of any one or more of β2m, TRAC, CIITA, RFXANK, RFXAP and RFX5 achieved by knockout or knockdown as described herein.

[0015] In another example, the present disclosure provides a population of engineered immune cells comprising an engineered immune cell provided herein. In an example, the population of engineered immune cells comprises between 10^4 and 10^{10} engineered immune

cells provided herein. In various examples, the population of engineered immune cells comprises 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, or 10^{10} engineered immune cells provided herein.

[0016] In another example, the present disclosure provides a population of engineered immune cells wherein no more than, for example, 75% of the cells functionally express one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK. In an example, no more than 75% of the cells of the population functionally express TAP2. In an example, no more than 75% of the cells of the population functionally express NLRC5. In an example, no more than 75% of the cells of the population functionally express RFX5.

[0017] In another example, the present disclosure provides a population of engineered immune cells wherein at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 75%, 90%, 95% or 100% of the engineered immune cells functionally express any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK at a reduced level. In some examples, the reduced level of expression, stated relative to an appropriate control e.g. the expression level in corresponding immune cells not correspondingly engineered, is 0%, for example when both chromosomal copies of a gene are knocked out (e.g. by methods that employ TALEN, zinc fingers, Cas-CLOVER, and/or CRISPR/Cas system), or 50%, for example when one of the two chromosomal copies of a gene is knocked out and there is no compensatory increase in expression of the other chromosomal copy of that gene. In some examples, the reduced level of expression, stated relative to the expression level in non-engineered immune cells, is between 0% and 90% or between any two values intermediate between 0% and 90%, for example between 10% and 90%, between 25% and 90%, between 25% and 75%, between 10% and 50%, between 25% and 50%, between 50% and 90%, and between 50% and 75%. Values within one or more of such intermediate ranges can be obtained when, for example, only one chromosomal copy of a gene is knocked out and a compensatory mechanism causes an increase in the level of expression of the remaining chromosomal copy, or reduction in expression is achieved by a method other than gene knockout, such as known knockdown methods e.g. those that employ any of various RNA-based techniques (e.g., shRNA anti-sense RNA, miRNA, siRNA; *see, e.g.*, Lam *et al.*, Mol. Ther.-Nucleic Acids 4:e252 (2015), doi:10.1038/mtna.2015.23; Sridharan and Gogtay, Brit. J. Clin. Pharmacol. 82: 659-72 (2016)). In some examples of the population of engineered immune cells disclosed herein, some or all of the engineered cells, e.g. 5-10%, 10-25%, 25-50%, 50-90%, or 90-100% exhibit

a reduced level of expression of an MHC class I protein and/or MHC class II protein or complex at the cell surface relative to a suitable control.

[0018] In various examples, the population of engineered immune cells or a population of immune cells comprising engineered immune cells as disclosed herein comprises at least 10% engineered T cells, at least 20% engineered T cells, at least 30% engineered T cells, at least 40% engineered T cells, at least 50% engineered T cells, at least 75% engineered T cells, at least 90% engineered T cells or 100% engineered T cells. Also provided herein is a population of cells of which at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 75%, at least 90% or 100% are engineered immune cells e.g. engineered T cells as disclosed herein.

[0019] In some examples, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 75%, or at least 90% of the engineered cells of the population further express an antigen binding protein. In some examples, the antigen binding protein is a chimeric antigen receptor (CAR). In some examples, a nucleic acid encoding the CAR is inserted into a disrupted TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP or RFXANK locus and/or such an insertion disrupts the locus. In some examples, the antigen binding protein is a T cell receptor (TCR). In some examples, a nucleic acid encoding the TCR is inserted into a disrupted TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP or RFXANK locus and/or such an insertion disrupts the locus.

[0020] In certain examples of the population of engineered immune cells disclosed herein, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 75%, or at least 90% of the engineered cells further comprises one or more genomic modifications of one or more of an endogenous TCRa gene and an endogenous CD52 gene.

[0021] In an example, the population of engineered immune cells is derived from one or more immune cells obtained from a person, for example, from a person other than the person to whom they will be administered, e.g. obtained from a donor other than the recipient or from a healthy volunteer, or is derived from one or more immune cells obtained from a patient e.g. the person to whom they will be administered, or is derived from one or more iPSCs.

[0022] In another example, the present disclosure provides a method of making the population of engineered immune cells described herein, wherein the method comprises the use of a gene editing technology, for example, a gene editing technology selected from the group consisting of TALEN, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system, to reduce functional

expression of any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK.

[0023] In another example, the present disclosure provides a method of determining or measuring the functional expression level of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and/or RFXANK in the cells of the population of engineered immune cells disclosed herein, wherein the functional expression level is measured by determining the surface expression level of an HLA protein, beta2 microglobulin (B2M) or both an HLA protein and B2M on the surface of the engineered immune cells, and/or is measured by flow cytometry.

[0024] In various examples of the engineered immune cell described herein and of the population of engineered immune cells disclosed herein, the engineered immune cell is, or one or more of the engineered immune cells of the population, e.g. at least 10%, 20%, 30%, 40%, 50%, 75%, 90% or 100% of the engineered immune cells of the population, are further engineered (e.g. by any of the methods disclosed herein or by any other method known to the person of ordinary skill in the art) to express one or more proteins selected from the group consisting of HLA-E, HLA-E single-chain trimer, HLA-G, HLA-G single-chain trimer, UL18, UL18 single-chain trimer, HLA-A2, HLA-A2 single-chain trimer, and human cytomegalovirus (HCMV) US11 by any method described herein or by any other method known to the person of ordinary skill in the art.

[0025] In another example, the present disclosure provides a pharmaceutical composition comprising an engineered immune cell as disclosed herein, wherein the composition further comprises one or more pharmaceutically acceptable carrier or excipient. In another example, the present disclosure provides a pharmaceutical composition comprising a population of engineered immune cells as disclosed herein, wherein the composition further comprises one or more pharmaceutically acceptable carrier or excipient. In various examples of the composition, the engineered immune cell or one or more of the engineered immune cells of the population, e.g. at least 10%, 20%, 30%, 40%, 50%, 75%, 90% or 100% of the engineered immune cells of the population, express(es) one or more proteins selected from the group consisting of HLA-E, HLA-E single-chain trimer, HLA-G, HLA-G single-chain trimer, UL18, UL18 single-chain trimer, HLA-A2, HLA-A2 single-chain trimer, and human cytomegalovirus (HCMV) US11, and/or do(es) not express or express(es) at a reduced level any one or more of CIITA, RFXANK, RFXAP and RFX5 achieved by knockout or knockdown as described herein.

[0026] In another example, the present disclosure provides a method of treating a condition in a patient comprising administering to the patient an engineered immune cell as disclosed herein, a population of engineered immune cells as disclosed herein, or a pharmaceutical composition as disclosed herein. In an example, the condition is selected from the group consisting of a solid tumor and a liquid tumor.

[0027] In another example, the present disclosure provides a method of decreasing the surface expression level of an MHC class I protein in an engineered immune cell, in some examples, to about 75% or less of the expression level of the MHC class I protein in non-engineered immune cells, the method comprising reducing the functional expression level of any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK, e.g. to about 75% or less of the expression level in non-engineered immune cells. In an example, the engineered immune cell functionally expresses TAP2 at a level not greater than 75% of the expression level in non-engineered immune cells. In an example, the engineered immune cell functionally expresses NLRC5 at a level not greater than 75% of the expression level in non-engineered immune cells. In an example, the engineered immune cell disclosed herein exhibits a reduced level of expression of an MHC class I protein or complex at the cell surface relative to a suitable control.

[0028] In an example, the engineered immune cell disclosed herein is an engineered T cell. In certain examples, the engineered immune cell disclosed herein, e.g. the engineered T cell disclosed herein, further expresses an additional protein e.g. a protein encoded by exogenous DNA or a protein whose expression is brought about by further engineering of the cell. In some examples, the additional protein is an antigen binding protein. In some examples, the antigen binding protein is a chimeric antigen receptor (CAR). In some examples, a nucleic acid encoding the additional protein, e.g. the antigen binding protein e.g. the CAR, is introduced into the cell by methods described herein. In some examples, the nucleic acid is introduced into a disrupted TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP or RFXANK locus or a TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP or RFXANK locus is disrupted by insertion of a nucleic acid encoding the additional protein e.g., the antigen binding protein, e.g. the CAR.

[0029] In an example of the method disclosed herein, the antigen binding protein is a T cell receptor (TCR) component, e.g. TCR α (TCR alpha), TCR β (TCR beta), TCR γ (TCR gamma) or TCR δ (TCR delta). In an example, a nucleic acid encoding the TCR

component is inserted into a disrupted TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP or RFXANK locus or a TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP or RFXANK locus is disrupted by insertion of a nucleic acid encoding the TCR component.

[0030] In a further example of the method disclosed herein, the engineered immune cell further comprises one or more genomic modifications (e.g. knock-out, deletion, knock-down, insertion) of one or more of an endogenous TCR alpha gene and an endogenous CD52 gene. In some examples, the genomic modification partially or wholly eliminates functional expression of the modified gene. In a further example of the method disclosed herein, the immune cell is an immune cell obtained from a person, e.g. from a donor other than the person to whom the cells will be administered, e.g. from a healthy volunteer, or is obtained from a patient e.g. the person to whom the cells will be administered, or is derived from an iPSC.

[0031] In another example, the present disclosure provides a method of making the population of engineered immune cells disclosed herein, wherein the method comprises the use of a gene editing technology, for example a gene editing technology selected from the group consisting of TALEN, zinc fingers, shRNA, Cas-CLOVER, and a CRISPR/Cas system, to reduce functional expression of any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK in a cell, e.g. in an immune cell. In an example, the gene editing technology introduces a mutation into one or more than one of a TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK genetic locus. In an example, the mutation is any one or more of an insertion, e.g. the insertion of one or more nucleotides or base pairs, e.g. the insertion of a sequence that encodes a protein, a deletion of one or more nucleotides or base pairs, and a substitution of one or more nucleotides or base pairs.

[0032] In an example of the method disclosed herein, the functional expression level of any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK is measured by determining the surface expression level of an HLA protein, of beta2 microglobulin (B2M) or of both HLA and B2M on the surface of the engineered immune cell, or is measured by flow cytometry. In an example, the extent of reduction in the surface expression level of one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK is determined relative to the corresponding expression level in a cell of the same type that has not been gene edited.

[0033] In an example of the methods disclosed herein, the engineered immune cell expresses an additional protein, which can be any desired protein including an antigen binding protein. In an example, the antigen binding protein comprises a CAR. In an example, a nucleic acid encoding the CAR is inserted into a disrupted TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP or RFXANK locus or such an insertion disrupts the locus. In another example, the antigen binding protein is a T cell receptor (TCR) component. In an example, a nucleic acid encoding the TCR is inserted into a disrupted TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP or RFXANK locus or such an insertion disrupts the locus. In an example, the method comprises reducing the functional expression level of the disrupted locus. In an example, the method comprises reducing the functional expression level of TAP2. In an example, the method comprises reducing the functional expression level of NLRC5. In an example, the method comprises reducing the functional expression level of RFX5. In an example, the method comprises reducing the functional expression level of CIITA. In certain examples, the method further comprises introducing into the engineered immune cell one or more genomic modifications of one or more of a gene encoding a TCR component e.g., a TCRa gene and a CD52 gene.

[0034] In another example, the present disclosure provides a method of reducing peptide diversity presented on the cell surface e.g. by MHC class I, the method comprising reducing the functional expression level of any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK, in some examples reducing this level to about 90% or less (in other words, a reduction of at least about 10%, e.g. to a level of about 90 or less compared to a control level of 100) or to about 75% or less of a comparable cell that has not been correspondingly altered. In various examples, reducing the functional expression level of one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK comprises the use of a gene editing technology selected from the group consisting of TALEN, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system (including for example, Cas9, Cas12 and MAD7). In an example, the method comprises reducing the functional expression level of TAP2. In an example, the method comprises reducing the functional expression level of NLRC5. In an example, the method comprises reducing the functional expression level of RFX5. In an example, the method comprises reducing the functional expression level of CIITA. In an example, the extent of reduction in the expression level of one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK is determined relative to the corresponding expression level in a cell of the same type that has not been gene

edited. In an example, the functional expression level of one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK is measured by determining the surface expression level of HLA, beta2 microglobulin (B2M) or both HLA and B2M on the surface of the engineered immune cell.

[0035] In another example, the present disclosure provides a method of decreasing T cell-mediated killing of allogeneic cells comprising reducing the functional expression level of any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK in an engineered immune cell such as an engineered T cell. In some examples, the method results in a reduced level of expression, stated relative to the expression level in non-engineered immune cells, that is 0%, for example when both chromosomal copies of a gene are knocked out, or 50%, for example when one of the two chromosomal copies of a gene is knocked out and there is no compensatory increase in expression of the other chromosomal copy of that gene. In some examples, the method results in a reduced level of expression, stated relative to the expression level in non-engineered immune cells, that is between 0% and 90% or between any two values intermediate between 0% and 90%, for example between 10% and 90%, between 25% and 90%, between 25% and 75%, between 10% and 50%, between 25% and 50%, between 50% and 90%, and between 50% and 75%. In some examples, reducing the functional expression level of one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK comprises the use of a gene editing technology, for example, any one or more of TALEN, zinc fingers, Cas-CLOVER, and/or CRISPR/Cas system, and/or any one or more known knockdown methods e.g. those that employ any of various RNA-based techniques (e.g., shRNA, anti-sense RNA, miRNA, siRNA). In an example, the method comprises reducing the functional expression level of TAP2. In an example, the method comprises reducing the functional expression level of NLRC5. In an example, the method comprises reducing the functional expression level of RFX5. In an example, the method comprises reducing the functional expression level of CIITA. In an example, the extent of reduction in the expression level of one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK is determined relative to the corresponding expression level in a cell of the same type that has not been so altered and/or manipulated.

[0036] In an example of the method of decreasing T cell-mediated killing of allogeneic cells disclosed herein, the functional expression level of one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK is measured by determining the surface

expression level of HLA, beta2 microglobulin (B2M) or both HLA and B2M on the surface of the engineered immune cell. In an example, the surface expression level of one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK is measured by flow cytometry. In an example, the method comprises introducing one or more genomic modifications of one or more of a gene encoding a TCR component e.g. a TCRa gene and a CD52 gene.

[0037] In some examples of the method of decreasing T cell-mediated killing of allogeneic cells disclosed herein, the functional expression level of any one or more of TAP2, NLRC5, β2m, TRAC, CIITA, RFX5, RFXAP and RFXANK in engineered immune cells of the present disclosure is assayed by measuring the degree to which the engineered immune cells survive in the presence of effector cells e.g. T cells, in comparison to the degree to which non-engineered, but otherwise comparable e.g. identical, immune cells survive under the same conditions.

[0038] In an example, the present disclosure provides an engineered immune cell that functionally expresses one or more of TAP2, NLRC5, β2m, TRAC, RFX5, RFXAP, CIITA and RFXANK at a reduced level. In various examples, TRAC and RFX5 are functionally expressed at a reduced level. In various examples, the cell exhibits a reduced level of expression of an MHC class I protein or complex at the cell surface, a reduced level of expression of an MHC class II protein or complex at the cell surface, or a reduced level of expression of an MHC class I protein or complex at the cell surface and a reduced level of expression of an MHC class II protein or complex at the cell surface. In various examples, the cell is a T cell.

[0039] In various examples, the engineered immune cell disclosed herein further expresses an additional protein. In some examples, the additional protein is an antigen binding protein. In some examples, the antigen binding protein is a chimeric antigen receptor (CAR). In some examples, the antigen binding protein is a T cell receptor (TCR). In certain examples, the engineered immune cell comprises a nucleic acid encoding the additional protein. In some examples, the nucleic acid encoding the additional protein is located within a disrupted TAP2, NLRC5, CIITA, RFX5, RFXANK, β2m or RFXAP locus and/or causes or creates a disruption in such a locus.

[0040] In various examples, the engineered immune cell disclosed herein comprises or further comprises one or more genomic modifications of one or more of an endogenous TCRa (TCRα or TCR alpha) gene and an endogenous CD52 gene.

[0041] In various examples, the engineered immune cell disclosed herein is or is derived from an immune cell obtained from a healthy volunteer or a patient, or is derived from an iPSC.

[0042] In an example, the present disclosure provides a method of making an engineered immune cell disclosed herein comprising the use of a gene editing technology selected from the group consisting of TALENs, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system to reduce functional expression of one or more of TAP2, NLRC5, β2m, TRAC, RFX5, RFXAP, CIITA and RFXANK.

[0042a] In one aspect, the present disclosure refers to an engineered immune cell wherein the cell is engineered so that one or more of RFX5, NLRC5, TAP2, β2m, TRAC, RFXAP, CIITA and RFXANK are functionally expressed at a reduced level.

[0042b] In another aspect, the present disclosure refers to a method of making the engineered immune cell as disclosed herein comprising the use of a gene editing technology selected from the group consisting of TALENs, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system to reduce functional expression of one or more of TAP2, NLRC5, β2m, TRAC, RFX5, RFXAP, CIITA and RFXANK.

[0042c] In another aspect, the present disclosure refers to an engineered immune cell that functionally expresses one or both of TAP2 and NLRC5 at a reduced level.

[0042d] In another aspect, the present disclosure refers to a method of making the engineered immune cell as disclosed herein comprising the use of a gene editing technology selected from the group consisting of TALENs, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system to reduce functional expression of one or both of TAP2 and NLRC5 in an immune cell.

[0042e] In another aspect, the present disclosure refers to a population of engineered immune cells wherein no more than 75% of the engineered immune cells functionally express one or both of TAP2 and NLRC5.

[0042f] In another aspect, the present disclosure refers to a population of engineered immune cells wherein at least 1% of the engineered immune cells functionally express one or both of

TAP2 and NLRC5 at a level not greater than 75% of the expression level in non-engineered immune cells.

[0042g] In another aspect, the present disclosure refers to a method of making the population of engineered immune cells as disclosed herein comprising the use of a gene editing technology selected from the group consisting of TALEN, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system to reduce functional expression of one or both of TAP2 and NLRC5.

[0042h] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the engineered immune cell as disclosed herein or the engineered immune cell made by the method as disclosed herein, or the population of engineered immune cells as disclosed herein, and further comprising at least one pharmaceutically acceptable carrier or excipient.

[0042i] In another aspect, the present disclosure refers to use of the engineered immune cell as disclosed herein or the engineered immune cell made by the method as disclosed herein, or the population of engineered immune cells as disclosed herein, or the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating a condition in a patient.

[0042j] In another aspect, the present disclosure refers to a method of decreasing the surface expression level of an endogenous MHC class I protein in an engineered immune cell to about 75% or less of the expression level of the endogenous MHC class I or MCH Class II protein in non-engineered immune cells, the method comprising reducing the functional expression level of one or both of TAP2 and NLRC5 to about 75% or less of the expression level in non-engineered immune cells.

[0042k] In another aspect, the present disclosure refers to a method of reducing peptide diversity presented by MHC class I in an immune cell comprising reducing the functional expression level of one or both of TAP2 and NLRC5 to about 75% or less of wild type level in the immune cell.

[0042l] In another aspect, the present disclosure refers to a method of decreasing T cell-mediated killing of allogeneic cells comprising reducing the functional expression level of one or both of TAP2 and NLRC5 to about 50% or less of the corresponding level in a non-engineered cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] FIG. 1 presents a non-comprehensive illustration of interactions between peptide-MHC complexes on an allogeneic CAR-T cell (center) and receptors on host T (top) and NK cells (left, right, bottom). Through the TCR, T cells kill foreign cells by recognizing non-self peptides presented by the MHC (labelled "HLA" in FIG. 1). Through myriad inhibitory receptors (KIR2DL and KIR3DL, NKG2A, LILRB1), NK cells recognize 'missing self' as the absence of MHC interactions. In FIG. 1, T cell killing is activated because the allogeneic cell's HLA complex presents a foreign antigen (narrow rectangle) and the T cell's TCR/CD3 complex recognizes the antigen as foreign. NK cell killing is not activated because there is no "missing self" because, as illustrated, the allogeneic cell presents the HLA complexes Classical HLA, HLA-E, and HLA-G (each of which displays a peptide).

[0044] FIGs. 2A-2B. T cells lacking surface MHC induce NK cell degranulation and are selectively killed. **FIG. 2A**: Quantification of flow cytometry data measuring degranulation of NK cells after 3 h incubation alone or with autologous or allogeneic T cells ± b2m KO in the presence of monensin (GolgiStop) and anti-CD107a antibody. **FIG. 2B**. Target composition over time of surface MHC+ (b2m WT) and surface MHC^{neg} (b2m KO) incubated with NK cells. The composition of b2m WT:b2m KO was 1:1 (50%:50%) at the start of the incubation. Mean + SD is shown for 3 experiments. WT, wild-type. KO, knockout.

[0045] FIG. 3 illustrates MHC class I biogenesis and peptide-loading pathways in nucleated mammalian cells. Multiple MHC-related genes (MHC class I, b2m, TAP, proteasomal components) are under transcriptional control of IFN-γ (IFN gamma)-inducible NLRC5. The

WHAT IS CLAIMED IS

1. An engineered immune cell wherein the cell is engineered so that one or more of RFX5, NLRC5, TAP2, β2m, TRAC, RFXAP, CIITA and RFXANK are functionally expressed at a reduced level.

2. The engineered immune cell of claim 1, wherein TRAC and RFX5 or TRAC and NLRC5 are functionally expressed at a reduced level.

3. The engineered immune cell of claim 1 or 2, wherein the cell exhibits:

a reduced level of expression of an MHC class I protein or complex at the cell surface,

a reduced level of expression of an MHC class II protein or complex at the cell surface, or

a reduced level of expression of an MHC class I protein or complex at the cell surface and a reduced level of expression of an MHC class II protein or complex at the cell surface.

4. The engineered immune cell of any one of claims 1-3, wherein the cell is a T cell.

5. The engineered immune cell of any one of claims 1-4, wherein the cell further expresses an antigen binding protein.

6. The engineered immune cell of claim 5, wherein the antigen binding protein is a chimeric antigen receptor (CAR).

7. The engineered immune cell of claim 5, wherein the antigen binding protein is a T cell receptor (TCR).

8. The engineered immune cell of any one of claims 5-7, wherein the engineered immune cell comprises a nucleic acid encoding the antigen binding protein.

9. The engineered immune cell of claim 8, wherein the nucleic acid encoding the antigen binding protein is located within a disrupted TAP2 or NLRC5 locus, or a locus selected from the group consisting of CIITA, RFX5, RFXANK and RFXAP.

10. The engineered immune cell of any one of claims 1-9, wherein the engineered immune cell further comprises one or more genomic modifications of one or more of an endogenous TCRa gene and an endogenous CD52 gene.

11. The engineered immune cell of any one of claims 1-10, wherein the immune cell is or is derived from an immune cell obtained from a healthy volunteer, is obtained from a patient, or is derived from an iPSC.

12. A method of making the engineered immune cell of any one of claims 1-11 comprising the use of a gene editing technology selected from the group consisting of TALENs, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system to reduce functional expression of one or more of TAP2, NLRC5, β2m, TRAC, RFX5, RFXAP, CIITA and RFXANK.

13. An engineered immune cell that functionally expresses one or both of TAP2 and NLRC5 at a reduced level.

14. The engineered immune cell of claim 13, wherein the cell comprises:

(a) a knockout at one or both alleles of TAP2,

(b) a knockout at one or both alleles of NLRC5, or

(c) a knockout at one or both alleles of TAP2 and a knockout at one or both alleles of NLRC5.

15. The engineered immune cell of claim 13 or 14, wherein the cell expresses one or both of TAP2 and NLRC5 at a level not greater than 90%, not greater than 75%, not greater than 50%, not greater than 25%, or not greater than 10% of the expression level in a non-engineered immune cell.

16. The engineered immune cell of any one of claims 13-15, wherein the cell functionally expresses TAP2 at a level not greater than 90%, not greater than 75%, not greater than 50%, not greater than 25%, or not greater than 10% of the expression level in a non-engineered immune cell.

17. The engineered immune cell of any one of claims 13-16, wherein the cell functionally expresses NLRC5 at a level not greater than 90%, not greater than 75%, not greater than 50%, not greater than 25%, or not greater than 10% of the expression level in a non-engineered immune cell.

18. The engineered immune cell of any one of claims 13-17, wherein the cell exhibits a reduced level of expression of an MHC class I protein or complex at the cell surface or an MHC class II protein or complex at the cell surface.

19. The engineered immune cell of any one of claims 13-18, wherein the cell is a T cell.

20. The engineered immune cell of any one of claims 13-19, wherein the cell further expresses an antigen binding protein.

21. The engineered immune cell of claim 20, wherein the antigen binding protein is a chimeric antigen receptor (CAR).

22. The engineered immune cell of claim 20, wherein the antigen binding protein is a T cell receptor (TCR).

23. The engineered immune cell of any one of claims 20-22, wherein the engineered immune cell comprises a nucleic acid encoding the antigen binding protein.

24. The engineered immune cell of claim 23, wherein the nucleic acid encoding the antigen binding protein is located within a disrupted TAP2 or NLRC5 locus, or a locus selected from the group consisting of CIITA, RFX5, RFXANK and RFXAP.

25. The engineered immune cell of any one of claims 13-24, wherein the engineered immune cell further comprises one or more genomic modifications of one or more of an endogenous TCRa gene and an endogenous CD52 gene.

26. The engineered immune cell of any one of claims 13-25, wherein the immune cell is or is derived from an immune cell obtained from a healthy volunteer, is obtained from a patient, or is derived from an iPSC.

27. A method of making the engineered immune cell of any one of claims 13-26 comprising the use of a gene editing technology selected from the group consisting of TALENs, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system to reduce functional expression of one or both of TAP2 and NLRC5 in an immune cell.

28. The engineered immune cell of any one of claims 13-26 or the engineered immune cell made by the method of claim 27, wherein the functional expression level of TAP2 and/or NLRC5 is measured by determining the surface expression level of HLA, beta2 microglobulin (β2M) or both HLA and β2M on the surface of the engineered immune cell, or is measured by flow cytometry.

29. The engineered immune cell of any one of claims 1-11, 13-26 or 28 or the engineered cell made by the method of claim 12 or 27, wherein:

(a) the engineered immune cell is further engineered to express one or more proteins selected from the group consisting of HLA-E, HLA-E single-chain trimer, HLA-G, HLA-G single-chain trimer, UL18, UL18 single-chain trimer, HLA-A2, HLA-A2 single-chain trimer, and human cytomegalovirus (HCMV) US11, and/or

(b) the engineered immune cell is further engineered to not express or to express at a reduced level any one or more of CIITA, RFXANK, RFXAP and RFX5.

30. A population of engineered immune cells wherein no more than 75% of the engineered immune cells functionally express one or both of TAP2 and NLRC5.

31. A population of engineered immune cells wherein at least 1% of the engineered immune cells functionally express one or both of TAP2 and NLRC5 at a level not greater than 75% of the expression level in non-engineered immune cells.

32. The population of engineered immune cells of claim 30, wherein no more than 75% of the cells functionally express TAP2.

33. The population of engineered immune cells of claim 30, wherein no more than 75% of the cells functionally express NLRC5.

34. The population of engineered immune cells of any one of claims 30-33, wherein at least 50% of the engineered immune cells exhibit a reduced level of expression of an MHC class I protein or complex at the cell surface.

35. The population of engineered immune cells of any one of claims 30-34, wherein the population of engineered immune cells comprises at least 10% engineered T cells, at least 20% engineered T cells, at least 30% engineered T cells, at least 40% engineered T cells, at least 50% engineered T cells, at least 75% engineered T cells, or at least 90% engineered T cells.

36. The population of engineered immune cells of any one of claims 30-35, wherein at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 75%, or at least 90% of the engineered cells further express an antigen binding protein.

37. The population of engineered immune cells of claim 36, wherein the antigen binding protein is a chimeric antigen receptor (CAR).

38. The population of engineered immune cells of claim 37, wherein a nucleic acid encoding the CAR is inserted into a disrupted TAP2 or NLRC5 locus or a locus selected from the group consisting of CIITA, RFX5, RFXANK and RFXAP.

39. The population of engineered immune cells of claim 36, wherein the antigen binding protein is a T cell receptor (TCR).

40. The population of engineered immune cells of claim 39, wherein a nucleic acid encoding the TCR is inserted into a disrupted TAP2 or NLRC5 locus or a locus selected from the group consisting of CIITA, RFX5, RFXANK and RFXAP.

41. The population of engineered immune cells of any one of claims 30-40, wherein at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 75%, or at least 90% of the engineered cells further comprises one or more genomic modifications of one or more of an endogenous TCRa gene and an endogenous CD52 gene.

42. The population of engineered immune cells of any one of claims 30-41, wherein the population of engineered immune cells is derived from one or more immune cells obtained

from a healthy volunteer, is derived from one or more immune cells obtained from a patient, or is derived from one or more iPSCs.

43. A method of making the population of engineered immune cells of any one of claims 30-42 comprising the use of a gene editing technology selected from the group consisting of TALEN, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system to reduce functional expression of one or both of TAP2 and NLRC5.

44. The population of engineered immune cells of any one of claims 30-42, wherein the functional expression level of TAP2 and/or NLRC5 is measured by determining the surface expression level of HLA, beta2 microglobulin (B2M) or both HLA and B2M on the surface of the engineered immune cell, or is measured by flow cytometry.

45. The population of engineered immune cells of any of claims 30-42 and 44, wherein at least 10%, 20%, 30%, 40%, 50%, 75%, or 90% of the engineered immune cells further express one or more proteins selected from the group consisting of HLA-E, HLA-E single-chain trimer, HLA-G or HLA-G single-chain trimer, UL18 or UL18 single-chain trimer, HLA-A2 and HLA-A2 single-chain trimer.

46. A pharmaceutical composition comprising the engineered immune cell of any one of claims 1-11, 13-26, and 28-29 or the engineered immune cell made by the method of claim 12 or 27, or the population of engineered immune cells of any one of claims 30-42, and 44-45, and further comprising at least one pharmaceutically acceptable carrier or excipient.

47. The pharmaceutical composition of claim 46, wherein the engineered immune cell or one or more cells of the population of engineered immune cells of the pharmaceutical composition further expresses one or more proteins selected from the group consisting of HLA-E, HLA-E single-chain trimer, HLA-G, HLA-G single-chain trimer, UL18, UL18 single-chain trimer, HLA-A2, HLA-A2 single-chain trimer, and human cytomegalovirus (HCMV) US11, and/or is further engineered to not express or to express at a reduced level any one or more of CIITA, RFXANK, RFXAP and RFX5.

48. Use of the engineered immune cell of any one of claims 1-11, 13-26, and 28-29 or the engineered immune cell made by the method of claim 12 or 27, or
the population of engineered immune cells of any one of claims 30-42, and 44-45, or
the pharmaceutical composition of claim 46 or 47, in the manufacture of a medicament for treating a condition in a patient.

49. The use of claim 48, wherein the condition is a solid tumor or a liquid tumor.

50. A method of decreasing the surface expression level of an endogenous MHC class I protein in an engineered immune cell to about 75% or less of the expression level of the endogenous MHC class I or MCH Class II protein in non-engineered immune cells, the method comprising reducing the functional expression level of one or both of TAP2 and NLRC5 to about 75% or less of the expression level in non-engineered immune cells.

51. The method of claim 50, wherein the cell functionally expresses TAP2 at a level not greater than 75% of the expression level in non-engineered immune cells.

52. The method of claim 50, wherein the cell functionally expresses NLRC5 at a level not greater than 75% of the expression level in non-engineered immune cells.

53. The method of any one of claims 50-52, wherein the engineered immune cell expresses an MHC class I protein.

54. The method of claim 53, wherein the engineered immune cell is a T cell.

55. The method of any one of claims 50-54, wherein the cell further expresses an antigen binding protein.

56. The method of claim 55, wherein the antigen binding protein is a chimeric antigen receptor (CAR).

57. The method of claim 56, wherein a nucleic acid encoding the CAR is inserted into a disrupted TAP2 or NLRC5 locus or wherein a TAP2 or NLRC5 locus is disrupted by insertion of a nucleic acid encoding the CAR.

58. The method of claim 55, wherein the antigen binding protein is a T cell receptor (TCR).

59. The method of claim 58, wherein a nucleic acid encoding the TCR is inserted into a disrupted TAP2 or NLRC5 locus or wherein a TAP2 or NLRC5 locus is disrupted by insertion of a nucleic acid encoding the TCR.

60. The method of any one of claims 50-59, wherein the engineered immune cell further comprises one or more genomic modifications of one or more of an endogenous TCRa gene and an endogenous CD52 gene.

61. The method of any one of claims 50-60, wherein the immune cell is an immune cell obtained from a healthy volunteer, is obtained from a patient, or is derived from an iPSC.

62. The method of any one of claims 50-61 comprising the use of a gene editing technology selected from the group consisting of TALEN, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system to reduce functional expression of one or both of TAP2 and NLRC5.

63. The method of any one of claims 50-62, wherein the functional expression level of TAP2 and/or NLRC5 is measured by determining the surface expression level of HLA, beta2

microglobulin (β2M) or both HLA and B2M on the surface of the engineered immune cell, or is measured by flow cytometry.

64. The method of any one of claims 50-63, wherein the extent of reduction in the surface expression level of one or more of TAP2 and NLRC5 is determined relative to the expression level of one or more of TAP2 and NLRC5, respectively, in a cell of the same type that has not been gene edited.

65. The method of any one of claims 50-64, wherein the engineered immune cell expresses an antigen binding protein.

66. The method of claim 65 wherein the antigen binding protein comprises a CAR.

67. The method of claim 66, wherein a nucleic acid encoding the CAR is inserted into a disrupted TAP2 or NLRC5 locus.

68. The method of claim 67, wherein the antigen binding protein is a T cell receptor (TCR).

69. The method of claim 68, wherein a nucleic acid encoding the TCR is inserted into a disrupted TAP2 or NLRC5 locus.

70. The method of any one of claims 64-69, wherein the method comprises reducing the functional expression level of TAP2.

71. The method of any one of claims 64-69, wherein the method comprises reducing the functional expression level of NLRC5.

72. The method of any one of claims 64-71, wherein the method further comprises introducing into the engineered immune cell one or more genomic modifications of one or more of a TCRa gene and a CD52 gene.

73. A method of reducing peptide diversity presented by MHC class I in an immune cell comprising reducing the functional expression level of one or both of TAP2 and NLRC5 to about 75% or less of wild type level in the immune cell.

74. The method of claim 73, wherein reducing the functional expression level of one or more of TAP2 and NLRC5 comprises the use of a gene editing technology selected from the group consisting of TALEN, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system to disrupt one or more of TAP2 and NLRC5.

75. The method of any one of claims 73-74, wherein the method comprises reducing the functional expression level of TAP2.

76. The method of any one of claims 73-74, wherein the method comprises reducing the functional expression level of NLRC5.

77. The method of any one of claims 73-76, wherein the extent of reduction in the expression level of one or more of TAP2 and NLRC5 is determined relative to the expression level of one or more of TAP2 and NLRC5, respectively, in a cell of the same type that has not been gene edited.

78. The method of any one of claims 73-77, wherein the functional expression level of TAP2 and NLRC5 is measured by determining the surface expression level of HLA, beta2 microglobulin (B2M) or both HLA and B2M on the surface of the engineered immune cell.

79. A method of decreasing T cell-mediated killing of allogeneic cells comprising reducing the functional expression level of one or both of TAP2 and NLRC5 to about 50% or less of the corresponding level in a non-engineered cell.

80. The method of claim 79, wherein reducing the functional expression level of one or more of TAP2 and NLRC5 comprises the use of a gene editing technology selected from the group consisting of TALEN, zinc fingers, Cas-CLOVER, and a CRISPR/Cas system.

81. The method of any one of claims 79-80, wherein the method comprises reducing the functional expression level of TAP2.

82. The method of any one of claims 79-81, wherein the method comprises reducing the functional expression level of NLRC5.

83. The method of any one of claims 79-82, wherein the extent of reduction in the expression level of one or more of TAP2 and NLRC5 is determined relative to the expression level of one or more of TAP2 and NLRC5, respectively, in a cell of the same type that has not been gene edited.

84. The method of any one of claims 79-83, wherein the functional expression level of one or more of TAP2 and NLRC5 is measured by determining the surface expression level of HLA, beta2 microglobulin (B2M) or both HLA and B2M on the surface of the engineered immune cell.

85. The method of claim 84, wherein the surface expression level of one or more of TAP2 and/or NLRC5 is measured by flow cytometry.

86. The method of any one of claims 79-85, wherein the method further comprises introducing one or more genomic modifications of one or more of a TCRa gene and a CD52 gene.