

MODIFIED CELLS AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to modified cells (e.g., modified immune cells), pharmaceutical compositions thereof, and methods of using such to prevent and/or treat diseases, cancer, viral infectious diseases, neurodegenerative diseases, autoimmune diseases, and the like, such as disease associated with auto-reactive lymphocytes, e.g., T cell mediated autoimmune disease.

BACKGROUND

[0002] Chimeric natural killer receptors (CNK) can redirect immune cells to specifically recognize and kill target cells expressing natural killer (NK) receptor ligands. CNKs are artificial multi-molecular proteins comprising an NK receptor domain and an NK signaling adaptor domain, such as those described, for example, in CNK-T and CNK-UT mentioned in "*Chimeric Natural Killer Cell Receptors and Method of Use Thereof* (Patent application no. 16/827697); *Universal T Cells and the Method of Use Thereof* (Patent application no. 17/164834) and the NKG2D-based CAR-T; *Chang et al., A Chimeric Receptor with NKG2D Specificity Enhances Natural Killer Cell Activation and Killing of Tumor Cells. Cancer Res; 73(6) March 15, 2013; Han et al., Control of triple-negative breast cancer using ex vivo self-enriched, costimulated NKG2D CAR T cells. Journal of Hematology & Oncology (2018) 11:92; Li et al., Human iPSC-derived Natural Killer Cells Engineered with Chimeric Antigen Receptors Enhance Anti-Tumor Activity. Cell Stem Cell. 2018 August 02; 23(2): 181–192.e5.; VanSeggelen et al., T Cells Engineered With Chimeric Antigen Receptors Targeting NKG2D Ligands Display Lethal Toxicity in Mice. Molecular Therapy vol. 23 no. 10 oct. 2015.*

[0003] However, existing immune cells expressing CNK or NK receptor-based CAR mentioned above showed poor proliferation capacity or therapeutic efficacy in treating diseases, especially in treating diseases associated with auto-reactive lymphocytes, e.g., autoimmune disease.

[0004] Therefore, there is a need for development of the novel NK receptor based adoptive cell therapies with increased therapeutic efficacies, especially in autoimmune diseases.

SUMMARY OF THE INVENTION

[0005] In one aspect, the present disclosure provides a novel design of CNK or NKG2D-based CAR via introduction of NK Protective Element (NK-PE) to suppress expression or

target degradation of NK ligand in T cells and protect the modified T cells from fratricide due to NK receptor and NK Ligands interaction.

[0006] In one aspect, the present disclosure provides a genetically modified mammalian immune cell or a population thereof, comprising a surface molecule and a protection element,

wherein the surface molecule is expressed on the surface of the immune cell, and is capable of binding to one or more target NK receptor ligand (tNKRL) expressed on a target cell thereby activating the immune cell to exert cytotoxic effect on the target cell,

wherein the immune cell, when activated, is capable of being induced to express one or more induced NK receptor ligand (iNKRL) that is capable of binding to the surface molecule, and

wherein the protection element substantially suppresses expression of the one or more induced NK receptor ligand on the immune cell surface following activation of the immune cell, thereby preventing the activated immune cell from becoming a target cell.

[0007] In certain embodiments, at least one of the target NK receptor ligands is identical to at least one of the induced NK receptor ligands, or wherein the surface molecule cross-reacts with at least one of the target NK receptor ligands and at least one of the induced NK receptor ligands.

[0008] In certain embodiments, the one or more target NK receptor ligand is selected from the group consisting of: NKG2D ligands, NCR ligands, KIR ligands, CD226 ligands, TIGIT ligands, CD96 ligands, or any combination thereof.

[0009] In certain embodiments, the one or more target NK receptor ligand comprises one or more NKG2D ligand.

[0010] In certain embodiments, the NKG2D ligand is selected from the group consisting of: MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6.

[0011] In certain embodiments, the one or more induced NK receptor ligand is selected from the group consisting of: NKG2D ligands, NCR ligands, KIR ligands, CD226 ligands, TIGIT ligands, CD96 ligands, or any combination thereof.

[0012] In certain embodiments, the one or more induced NK receptor ligand comprises one or more NKG2D ligand.

[0013] In certain embodiments, the NKG2D ligand is selected from the group consisting of: MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6.

[0014] In certain embodiments, the at least one of the target NK receptor ligands is identical to at least one of the induced NK receptor ligands and comprises MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6.

[0015] In certain embodiments, the expression of the one or more induced NK receptor ligand on the immune cell surface is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%) relative to expression of the one or more induced NK receptor ligand on a reference immune cell surface without the protection element.

[0016] In certain embodiments, the expression of the one or more induced NK receptor ligand on the immune cell surface is reduced such that the binding the one or more induced NK receptor ligand to the surface molecule is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%).

[0017] In certain embodiments, the expression of the one or more induced NK receptor ligand is detectable on no more than 50% (e.g., no more than 40%, no more than 30%, no more than 20%, or no more than 10%) of the population of the immune cells following binding of the surface molecule to the one or more target NK receptor ligand.

[0018] In certain embodiments, the one or more induced NK receptor ligand comprises MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6 and the expression level of the MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6 on the immune cell surface is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%).

[0019] In certain embodiments, the one or more induced NK receptor ligand comprises MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP5 and ULBP6 and the expression level of each of the MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP5 and ULBP6 on the immune cell surface is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%).

[0020] In certain embodiments, the immune cell has substantially reduced activation-induced cell death of the immune cell, optionally the reduced activation-induced cell death of the immune cell can be determined by reduced apoptosis detected by Annexin V/7-AAD.

[0021] In certain embodiments, the immune cell is capable of proliferate upon activation (e.g., having an increased proliferation rate as compared to a modified mammalian immune cell without the protection element).

[0022] In certain embodiments, the protection element is capable of silencing expression of the one or more induced NK receptor ligand or is capable of inducing intracellular retention and/or degradation of the one or more induced NK receptor ligand.

[0023] In certain embodiments, the protection element comprises a small RNA (e.g., an siRNA or miRNA or sh RNA) that targets mRNAs of the one or more induced NK receptor ligand.

[0024] In certain embodiments, the protection element comprises one or more protein degradation element that is capable of inducing intracellular retention and/or degradation of the one or more induced NK receptor ligand.

[0025] In certain embodiments, at least one of (or all of) the protein degradation elements comprise an endoplasmic reticulum (ER)-retention domain and an iNKRL binding domain capable of binding to the one or more induced NK receptor ligand.

[0026] In certain embodiments, the protein degradation element further comprises an ER-retention transmembrane domain, wherein the ER-retention transmembrane domain has one end operably linked to the iNKRL binding domain and the other end operably linked to the ER retention domain.

[0027] In certain embodiments, the ER-retention domain and/or the ER-retention transmembrane domain is derived from an ER-retention virus protein.

[0028] In certain embodiments, the ER-retention virus protein is selected from the group consisting of: HCMV protein US2, HCMV protein US11, HCMV protein US18, HCMV protein US20, HCMV protein US3, HCMV protein US10, HCMV protein US6, HSV ICP47, CPXV12, BHV UL49.5, EBV BNFL2a, HCMV protein UL16, HCMV protein UL141, HCMV protein UL142, HIV Nef, HIV Vpu, HHV-7 U21, HHV-8 KK3, HHV-8 KK5, MHV-68 MK3, HTLV-1 p12, Cowpox Virusprotein CPXV203 and Ad E3/19K.

[0029] In certain embodiments, the ER-retention domain and the ER-retention transmembrane domain are both derived from the same ER-retention virus protein.

[0030] In certain embodiments, the iNKRL binding domain comprises an antibody domain (e.g., scFv) capable of binding to the one or more induced NK receptor ligand.

[0031] In certain embodiments, the iNKRL binding domain is derived from a virus protein capable of binding to the one or more induced NK receptor ligand.

[0032] In certain embodiments, the one or more induced NK receptor ligand comprises one or more NKG2D ligands.

[0033] In certain embodiments, the one or more NKG2D ligands are selected from the group consisting of: MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

[0034] In certain embodiments, the virus protein capable of binding to the one or more NK receptor ligand is derived from a virus selected from the group consisting of: adenovirus (Ad) (e.g., human Ad), cytomegalovirus (CMV) (human CMV, i.e., HCMV) and Kaposi's sarcoma-associated herpesvirus (KSHV).

[0035] In certain embodiments, the virus protein capable of binding to the one or more NK receptor ligand is derived from a virus protein selected from the group consisting of: Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), HCMV protein UL16, HCMV protein UL142, HCMV protein US18, HCMV protein US20, and KSHV K5.

[0036] In certain embodiments, the ER-retention domain and the binding domain are both derived from the same virus protein.

[0037] In certain embodiments, the ER-retention domain, the ER-retention transmembrane domain and the binding domain are all derived from the same virus protein.

[0038] In certain embodiments, the same virus protein is selected from the group consisting of Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), HCMV protein UL16, HCMV protein UL142, HCMV protein US18, HCMV protein US20, KSHV K5.

[0039] In certain embodiments, the protein degradation element comprises a virus protein selected from the group consisting of: Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), HCMV protein UL16, HCMV protein UL142, HCMV protein US18, HCMV protein US20, KSHV K5 or an analog thereof, or a derivative thereof.

[0040] In certain embodiments, the protection element comprises a small RNA, such as HCMV encoded microRNAs (miR-20a, miR-UL112) are able to modulate NKG2D-ligand expression.

[0041] In certain embodiments, the one or more protein degradation elements comprise a combination of virus proteins, wherein the combination of virus proteins comprises a HCMV protein UL16 and another virus protein selected from the group consisting of: Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), HCMV protein UL142, HCMV protein US18, HCMV protein US20, and KSHV K5.

[0042] In certain embodiments, the one or more protein degradation elements further comprise a protein degradation pathway (PDP) binding domain capable of binding to a member in a protein degradation pathway.

[0043] In certain embodiments, the PDP binding domain is operably linked to the ER-retention domain.

[0044] In certain embodiments, the protein degradation pathway is ubiquitylation-proteasome pathway, endosome-lysosome pathway, or autophagic degradation pathway.

[0045] In certain embodiments, the member in the ubiquitylation-proteasome pathway comprises E1 ubiquitin activating enzyme, E2 ubiquitin conjugating enzyme, or E3 ubiquitin ligase.

[0046] In certain embodiments, the member in the endosome-lysosome pathway comprises AP-1, AP-2, AP-3, endosome, lysosome, HOPS, ESCRT, GASP, BLOC-1, ESCRT, Retromer, ESCRT, sortingnexin, Dapper2, SNX4, Pincher, Rap1-PDZ-GEF1, clathrin, or C3G/CrkL/Shp2/Gab2.

[0047] In certain embodiments, the member in the autophagic degradation pathway comprises chaperone-mediated autophagy (CMA) USP10, G3BP1, ULK1, ATG16L1, TRIM16, FBXO27VDAC, RHOT1, MFN1/2, BNIP3L, FUNDC1, BNIP3, AMBRA1, BCL2L13, FKBP8, CHDH, DISC1, PHB2, Cardiolipin, SEC62, RTN3, PEX5, PEX14, ABCD3, or NUFIP1.

[0048] In certain embodiments, the surface molecule comprises a) a tNKRL-binding extracellular domain capable of binding to the one or more target NK receptor ligand, and b) a surface molecule (SM) intracellular domain capable of activating the immune cell following binding of the tNKRL-binding extracellular domain to the one or more target NK receptor ligand, and wherein the tNKRL-binding extracellular domain is associated with the SM intracellular domain to allow transduction of binding signal from the tNKRL-binding extracellular domain to the SM intracellular domain.

[0049] In certain embodiments, the tNKRL-binding extracellular domain comprises an antibody domain that is capable of binding to the one or more target NK receptor ligand.

[0050] In certain embodiments, the tNKRL-binding extracellular domain comprises an NK receptor extracellular domain that is capable of binding to the one or more target NK receptor ligand.

[0051] In certain embodiments, the tNKRL-binding extracellular domain comprises an NKG2D extracellular domain.

[0052] In certain embodiments, the receptor further comprises a SM transmembrane domain, wherein the SM transmembrane domain has one end operably linked to the tNKRL-binding extracellular domain and the other end operably linked to the SM intracellular domain.

[0053] In certain embodiments, the SM transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of CD3, CD4, CD8, CD28, B7, ICOS, CD226, 41-BB, OX40, CD27, GITR, HVEM, CD40, BAFFR, BAFF, NKG2D, NKG2C, NKG2A, NKp44, NKp46, NKp30.

[0054] In certain embodiments, the surface molecule comprises a tNKRL-binding chimeric antigen receptor (CAR).

[0055] In certain embodiments, the receptor comprises an CNK receptor protein complex comprising a first polypeptide comprising the tNKRL-binding extracellular domain operably linked to a first CNK transmembrane domain, and a second polypeptide comprising the CNK intracellular domain operably linked to a second CNK transmembrane domain, wherein the first CNK transmembrane domain and the second CNK transmembrane domain associate with each other to form the protein complex.

[0056] In certain embodiments, the first CNK transmembrane domain comprises a NK receptor transmembrane domain, optionally an NKG2D transmembrane domain.

[0057] In certain embodiments, the first transmembrane domain is capable of forming a first dimer.

[0058] In certain embodiments, the first polypeptide comprises a NKG2D protein.

[0059] In certain embodiments, the NKG2D protein comprises the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2.

[0060] In certain embodiments, the second transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of DNAX-Activating Protein of 10 kDa (DAP10) and DNAX-Activating Protein of 12 kDa (DAP12).

[0061] In certain embodiments, the CNK intracellular domain comprises a cytoplasmic domain of a protein selected from the group consisting of: 4-1BB (CD137), CD2, CD3, CD35, CD25, CD27, CD28, CD30, CD40, CD79A, CD79B, CARD11, DAP10, DAP12, Fc receptor, Fyn, LIGHT, LT β R, HVEM, ICOS, Lck, LAG3, LAT, LRP, NOTCH1, NOTCH2, NOTCH3, NOTCH4, OX40 (CD134), ROR2, Ryk, SLAMF1, Slp76, pTa, TCR α , vTCRP, TRIM, Zap70, PTCH2, IL7 receptor, IL15 receptor and GITR.

[0062] In certain embodiments, the CNK intracellular domain comprises a cytoplasmic domain of CD3 (e.g., CD3 zeta).

[0063] In certain embodiments, the CNK intracellular domain comprises the amino acid sequence of SEQ ID NO: 5.

[0064] In certain embodiments, the second polypeptide comprises a chimeric adaptor protein comprising: the transmembrane domain of DAP10 operably linked to the intracellular domain of CD3 (e.g., CD3 zeta); the transmembrane domain of DAP12 operably linked to the intracellular domain of CD3 (e.g., CD3 zeta); or the transmembrane domain of DAP10 operably linked to the intracellular domain of DAP12.

[0065] In certain embodiments, the second polypeptide comprises a full-length DAP10 operably linked to the intracellular domain of CD3 zeta.

[0066] In certain embodiments, the chimeric adaptor protein comprises the amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 16.

[0067] In certain embodiments, the second CNK transmembrane domain is capable of forming a second dimer, and wherein the second dimer associates with the first CNK transmembrane domain to form the complex.

[0068] In certain embodiments, the CNK receptor comprises a hexameric protein complex comprising a homodimer of the NKG2D protein and two homodimers of the chimeric adaptor protein.

[0069] In certain embodiments, the immune cell is further modified to be deficient in a) MHC class I protein, or b) MHC class II protein or MHC class II transactivator, or c) both a) and b).

[0070] In certain embodiments, the immune cell is T cell and the immune cell is further modified to be deficient in endogenous TCR.

[0071] In certain embodiments, the genetically modified mammalian immune cell provided herein further expresses a target binding (TB) receptor capable of binding to a target marker.

- [0072]** In certain embodiments, the target marker is expressed on the target cell.
- [0073]** In certain embodiments, the TB receptor comprises a target-marker binding extracellular domain capable of binding to the target marker and a TB intracellular domain capable of activating the immune cell upon binding of the target-marker binding extracellular domain to the target marker.
- [0074]** In certain embodiments, the TB receptor further comprises a TB transmembrane domain.
- [0075]** In certain embodiments, the TB transmembrane domain has one end operably linked to the target-marker binding extracellular domain and the other end operably linked to the TB intracellular domain.
- [0076]** In certain embodiments, the target-marker binding extracellular domain comprises the extracellular domain of a natural cytotoxicity receptor (e.g., NKp30 (NCR3), NKp44 (NCR2), and NKp46 (NCR1)).
- [0077]** In certain embodiments, the TB transmembrane domain comprises the transmembrane domain of a protein selected from the group consisting of CD3, CD4, CD8, CD28, B7, ICOS, CD226, 41-BB, OX40, CD27, GITR, HVEM, CD40, BAFFR, BAFF, NKG2D, NKG2C, NKG2A, NKp44, NKp46, NKp30.
- [0078]** In certain embodiments, the TB intracellular domain comprises a cytoplasmic domain of a protein selected from the group consisting of: 4-1BB (CD137), CD2, CD3, CD35, CD25, CD27, CD28, CD30, CD40, CD79A, CD79B, CARD11, DAP10, DAP12, Fc receptor, Fyn, LIGHT, LT β R, HVEM, ICOS, Lck, LAG3, LAT, LRP, NOTCH1, NOTCH2, NOTCH3, NOTCH4, OX40 (CD134), ROR2, Ryk, SLAMF1, Slp76, pTa, TCRA, vTCRP, TRIM, Zap70, PTCH2, IL7 receptor, IL15 receptor and GITR.
- [0079]** In certain embodiments, the TB intracellular domain comprises a cytoplasmic domain of CD28 operably linked to CD3 zeta chain.
- [0080]** In certain embodiments, the target marker is a tumor antigen, an auto-reactive cell antigen or a virus antigen, bacteria antigen, a microbe-infected cell, or damaged and/or aged cells.
- [0081]** In certain embodiments, the target cell is a tumor cell, an auto-reactive cell, a senescent cell or a virus-infected cell.
- [0082]** In certain embodiments, the immune cell is a T cell, a Natural Killer (NK) cell, a NKT cell, a B cell, a hematopoietic cell (e.g., bone marrow cell), a thymocyte, a dendritic cell (e.g.,

a mature dendritic cell), a macrophage cell, a tumor infiltrating lymphocyte, a monocyte, a granulocyte.

[0083] In certain embodiments, the immune cell is a T cell, such as CD4⁺ T cell, CD8⁺ T cell, cytotoxic T cell, terminal effector T cell, memory T cell, naïve T cell, natural killer T cell, gamma-delta T cell, cytokine-induced killer (CIK) T cell, or tumor infiltrating lymphocyte.

[0084] In another aspect, the present disclosure provides a pharmaceutical composition comprising the genetically modified mammalian immune cell or the population thereof provided herein.

[0085] In another aspect, the present disclosure provides a method for treating a disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition provided herein.

[0086] In certain embodiments, the disease is cancer, a disease associated with auto-reactive lymphocytes (e.g., an autoimmune disease), an immune deficient disease, or an aging associated disease.

[0087] In certain embodiments, the autoimmune disease is organ-specific autoimmune disease or systemic autoimmune disease.

[0088] In certain embodiments, the organ-specific autoimmune disease is selected from the group consisting of chronic lymphocytic thyroiditis, hyperthyroidism, insulin-dependent diabetes mellitus, myasthenia gravis, ulcerative colitis, pernicious anemia with chronic atrophic gastritis, pulmonary hemorrhage nephritic syndrome, pemphigus vulgaris, pemphigoid, primary biliary cirrhosis, multiple sclerosis, acute idiopathic polyneuritis, etc.

[0089] In certain embodiments, the systemic autoimmune disease is selected from the group consisting of systemic lupus erythematosus, rheumatoid arthritis, systemic vasculitis, scleroderma, pemphigus, dermatomyositis, mixed connective tissue disease, autoimmune hemolytic anemia, thyroid autoimmune disease, and ulcerative colitis.

[0090] In certain embodiments, the autoimmune disease is Graft versus host disease (GvHD).

[0091] In another aspect, the present disclosure provides an expression construct encoding a surface molecule and a protection element, wherein the construct can be expressed in an immune cell, wherein the surface molecule is as defined as above, and the protection element is as defined as above.

[0092] In another aspect, the present disclosure provides a method of producing the genetically modified mammalian immune cell provided herein, comprising introducing the expression construct provided herein to a starting immune cell under conditions to allow expression of the surface molecule on the surface of the immune cell and to allow expression of the protection element in the immune cell.

BRIEF DESCRIPTION OF THE FIGURES

[0093] Figures 1A and 1B show T cell activation significantly upregulated expression of NKG2D ligands (e.g., MICA, MICB, ULBP-1, ULBP-2, 5, 6, ULBP-3).

[0094] Figure 2A. Schematic diagram illustrating the structure of lentiviral vectors. (1) The CNK vector contains the EF1a promotor, DAP10-CD3Z and NKG2D separated by self-cleavage sequence T2A; (2) The CNK+PE vector contains the EF1a promotor, DAP10-CD3Z, NKG2D and UL16 separated by self-cleavage sequence T2A ; (3) The CNK+PE/NCR2-CD28-CD3Z (i.e., CNK+PE/NCR2 ED-CD28 TM-CD28 ICD-CD3Z ICD) vector 1 contains the EF1a promotor, DAP10-CD3Z, NKG2D and UL16 separated by self-cleavage sequence T2A ; Vector 2 contains the EF1a promotor, NCR2 ED-CD28-CD3Z.

[0095] Figure 2B Phenotyping of different CNK-T cells with or without protection elements.

[0096] Figure 3 shows that the protection element (PE) could inhibit NKG2D ligand expression on the CNK-T cells.

[0097] Figure 4 shows that the protection element (PE) could increase CNK-T cell viability and proliferation.

[0098] Figure 5 shows phenotype of NCR2 CNK-UT cells.

[0099] Figure 6 shows CNK-UT efficiently eliminated activated T cells.

[00100] Figure 7 shows CNK-UT selectively killed activated T cells over inactivated T cells.

[00101] Figure 8 shows Jurkat T cells express NKG2D ligands and NCR ligands.

[00102] Figure 9 shows NCR2 CNK-UT cells efficiently kill Jurkat T cells. Figure 9A. shows flow cytometry plot of co-culture in different E:T ratio (1:2, 1:1, 2:1); Figure 9B shows the residual tumor cells (CD3+, CD8-) Jurkat T cells after 48h coculture based on the flow cytometry.

[00103] Figure 10 shows schematic diagram illustrating the mechanism of CNK-UT to treat T-cell mediated autoimmune diseases.

[00104] Figure 11 shows schematic illustration of GVHD mouse model experiment plan.

[00105] Figures 12A-12D are the representative FACS plots showing the percentage of human CD45+ and CD3+ T cells in PBL of GVHD mice treated with CNK-UT002 on day 11 (FIG. 12A), day 18 (FIG. 12B), day 25 (FIG. 12C) and day 32 (FIG. 12D), respectively.

[00106] Figure 13 shows the percentage of the donor T cells in the GVHD mouse model. The GVHD mice without treatment were used as a control. Data were representative plots from 8 mice.

[00107] Figure 14 shows GVHD score of the mice with or without treatment after allogeneic PBMC infusion.

[00108] Figure 15 shows the weight change of GVHD mice over the time.

[00109] Figure 16 shows the survival of percentage of GVHD mice over the time.

DETAILED DESCRIPTION

[00110] The following description of the invention is merely intended to illustrate various embodiments of the invention. As such, the specific modifications discussed are not to be construed as limitations on the scope of the invention. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is understood that such equivalent embodiments are to be included herein. All references cited herein, including publications, patents and patent applications are incorporated herein by reference in their entirety.

[00111] Although various technical features of the present disclosure are described in a single embodiment, the features can also be provided separately and/or in any suitable combination. Conversely, although various technical features of the present disclosure are described herein in separate embodiments for clarity, any of these features can also be implemented in a single embodiment.

[00112] Definitions

[00113] As used herein, the terms or terminology are for the purpose of describing particular embodiments only and are not intended to be limiting.

[00114] The singular terms “a”, “an”, and “the” include plural referents unless context clearly indicates otherwise. By way of example, reference to “a cell” refers to one or more cells, and reference to “the method” includes reference to equivalent steps and methods disclosed herein and/or known to those skilled in the art, and so forth. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The abbreviation, “e.g.,” is derived from the Latin *exempli gratia*, and is used herein to indicate a non-limiting example. Thus, the abbreviation “e.g.” is synonymous with the term “for example”.

[00115] In all occurrences in this application where there are a series of recited numerical values, it is to be understood that any of the recited numerical values may be the upper limit or lower limit of a numerical range. It is to be further understood that the invention encompasses all such numerical ranges, i.e., a range having a combination of an upper numerical limit and a lower numerical limit, wherein the numerical value for each of the upper limit and the lower limit can be any numerical value recited herein. Ranges provided herein are understood to include all values within the range. For example, 1-10 is understood to include all of the values 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, and fractional values as appropriate. Similarly, ranges delimited by “at least” are understood to include the lower value provided and all higher numbers.

[00116] As used herein, “about” is understood to include within three standard deviations of the mean or within standard ranges of tolerance in the specific art. In certain embodiments, about is understood to include a variation of no more than 0.5.

[00117] The term “including” is used herein to mean, and is used interchangeably with, the phrase “including but not limited to”. Similarly, “such as” is used herein to mean, and is used interchangeably, with the phrase “such as but not limited to”.

[00118] The term “comprising” or “comprises”, as used herein, is used in reference to compositions, methods, and respective component(s) thereof, that are essential to the method or composition, yet open to the inclusion of unspecified elements, whether essential or not.

[00119] The term “consisting of” refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[00120] As used herein, the term “substantially suppressing” in connection with the expression level of a protein refers to reduction in the expression level of at least 1%, at least 5%, at

least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% after suppressing.

[00121] The terms “polypeptide”, “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms also apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally-occurring amino acid, as well as to naturally-occurring amino acid polymers and non-naturally occurring amino acid polymers.

[00122] The term “nucleotide”, “nucleic acid” or “polynucleotide” as used herein includes oligonucleotides (i.e., short polynucleotides). They also refer to synthetic and/or non-naturally occurring nucleic acid molecules (e.g., comprising nucleotide analogues or modified backbone residues or linkages). The terms also refer to deoxyribonucleotide or ribonucleotide oligonucleotides in either single-or double-stranded form. The terms encompass nucleic acids containing analogues of natural nucleotides. The terms also encompass nucleic acid-like structures with synthetic backbones. Unless otherwise indicated, a particular polynucleotide sequence also implicitly encompasses conservatively modified variants thereof (e.g. degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (see Batzer *et al.*, *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260:2605-2608 (1985); and Rossolini *et al.*, *Mol. Cell. Probes* 8:91-98 (1994)).

[00123] A “conservative substitution” with reference to amino acid sequence refers to replacing an amino acid residue with a different amino acid residue having a side chain with similar physiochemical properties. For example, conservative substitutions can be made among amino acid residues with hydrophobic side chains (e.g. Met, Ala, Val, Leu, and Ile), among residues with neutral hydrophilic side chains (e.g. Cys, Ser, Thr, Asn and Gln), among residues with acidic side chains (e.g. Asp, Glu), among amino acids with basic side chains (e.g. His, Lys, and Arg), or among residues with aromatic side chains (e.g. Trp, Tyr, and Phe). As known in the art, conservative substitution usually does not cause significant change in the protein conformational structure, and therefore could retain the biological activity of a protein.

[00124] The term “functional equivalents” as used herein, refers to different forms (such as variants, fragments, fusions, derivatives and mimetics) of the parent molecule, which, despite of having difference in amino acid sequences or in chemical structures, still retains substantial biological activity of the parent molecule. The expression “retain substantial biological activity”, as used herein, means exhibiting at least part of (for example, no less than about 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) or all of the biological activity of the parent molecule. A functional equivalent of a parent protein may include both naturally-occurring variant forms and non-naturally occurring forms such as those obtained by recombinant methods or chemical synthesis. The functional equivalents may contain non-natural amino acid residues.

[00125] As used herein, the term “deficient” refers to insufficiency in activity or level, and can include, for example, being less than normal activity or level, or being absent or null in activity or level.

[00126] As used herein, the term “CAR”, which can be used interchangeably with the term "chimeric antigen receptor", refers to an engineered receptor or an artificial receptor or polynucleotide encoding thereof. The engineered receptor or a synthetic receptor comprises an extracellular domain that comprises an antigen binding domain, a transmembrane domain, and/or an intracellular signaling domain, which are joined one another or operably linked to each other, and confers specificity for an antigen onto an immune effector cell and bypasses MHC class I and class II restriction.

[00127] The term “chimeric antigen receptor T cell”, used interchangeably with the term “CAR-T cell” or “CART cell”, refers to a T cell or population thereof that has been engineered (e.g., through genetic engineering) to express a CAR on the T cell surface. CAR-T cells can be T helper CD4⁺ and/or T effector CD8⁺ cells. CAR-T cells can bind to a target cell expressing a target antigen and initiate immune response against the target cell.

[00128] As used herein, the term “TCR”, which can be used interchangeably with the term "T cell receptor" or the term “TCR complex” refers to a natural (or endogenous) TCR or an engineered TCR. TCR refers to a disulfide-linked membrane-anchored heterodimeric protein complex normally comprising an alpha chain and a beta chain that are highly variable, that are complexed with a CD3 γ , a CD3 δ , and two CD3 ϵ chains and CD3-zeta chains. The amino acid sequence of the alpha chain and beta chain varies among different T cells. The transmembrane regions of the alpha chain and the beta chain are enclosed by the CD3 transmembrane regions in an open barrel. The intracellular tails of the CD3 γ , CD3 δ and CD3 ϵ molecules each contain a single

conserved motif called immunoreceptor tyrosine-based activation motif (ITAM), which is critical for the signaling capacity of the TCR complex and to initiate signal transduction upon binding to antigen-MHC complex.

[00129] As used herein, the term “antigen” refers to a molecule that can be specifically recognized and bound by an antibody. One skilled in the art would understand that an antigen may be any large molecules or small molecules, and that almost all the proteins or peptides may be used as an antigen.

[00130] The term “antibody” as used herein includes any immunoglobulin, monoclonal antibody, polyclonal antibody, multivalent antibody, bivalent antibody, monovalent antibody, multispecific antibody, or bispecific antibody that binds to a specific antigen. A native intact antibody comprises two heavy (H) chains and two light (L) chains. Mammalian heavy chains are classified as alpha, delta, epsilon, gamma, and mu, each heavy chain consists of a variable region (V_H) and a first, second, and third constant region (C_{H1} , C_{H2} , C_{H3} , respectively); mammalian light chains are classified as λ or κ , while each light chain consists of a variable region (V_L) and a constant region. The antibody has a “Y” shape, with the stem of the Y consisting of the second and third constant regions of two heavy chains bound together via disulfide bonding. Each arm of the Y includes the variable region and first constant region of a single heavy chain bound to the variable and constant regions of a single light chain. The variable regions of the light and heavy chains are responsible for antigen binding. The variable regions in both chains generally contain three highly variable loops called the complementarity determining regions (CDRs) (light chain CDRs including LCDR1, LCDR2, and LCDR3, heavy chain CDRs including HCDR1, HCDR2, HCDR3). CDR boundaries for the antibodies and antigen-binding domains disclosed herein may be defined or identified by the conventions of Kabat, IMGT, AbM, Chothia, or Al-Lazikani (Al-Lazikani, B., Chothia, C., Lesk, A. M., J. Mol. Biol., 273(4), 927 (1997); Chothia, C. et al., J Mol Biol. Dec 5;186(3):651-63 (1985); Chothia, C. and Lesk, A.M., J.Mol.Biol., 196,901 (1987); N. R. Whitelegg et al, Protein Engineering, v13(12), 819-824 (2000); Chothia, C. et al., Nature. Dec 21-28;342(6252):877-83 (1989); Kabat E.A. et al., National Institutes of Health, Bethesda, Md. (1991); Marie-Paule Lefranc et al, Developmental and Comparative Immunology, 27: 55-77 (2003); Marie-Paule Lefranc et al, Immunome Research, 1(3), (2005); Marie-Paule Lefranc, Molecular Biology of B cells (second edition), chapter 26, 481-514, (2015)). The three CDRs are interposed between flanking stretches known as framework regions (FRs), which are more highly conserved than the CDRs and form a scaffold to support the hypervariable loops. The constant regions of the heavy and light chains are not involved in antigen-binding but exhibit various effector functions. Antibodies

are assigned to classes based on the amino acid sequence of the constant region of their heavy chain. The five major classes or isotypes of antibodies are IgA, IgD, IgE, IgG, and IgM, which are characterized by the presence of alpha, delta, epsilon, gamma, and mu heavy chains, respectively. Several of the major antibody classes are divided into subclasses such as IgG1 (gamma1 heavy chain), IgG2 (gamma2 heavy chain), IgG3 (gamma3 heavy chain), IgG4 (gamma4 heavy chain), IgA1 (alpha1 heavy chain), or IgA2 (alpha2 heavy chain). In certain embodiments, the antibody provided herein encompasses any antigen-binding fragments thereof.

[00131] As used herein, the term “antigen-binding fragment” refers to an antibody fragment formed from a fragment of an antibody comprising one or more CDRs, or any other antibody portion that binds to an antigen but does not comprise an intact native antibody structure. Examples of antigen-binding fragment include, without limitation, a diabody, a Fab, a Fab', a F(ab')₂, a Fd, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)₂, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multispecific antibody, a camelized single domain antibody, a nanobody, a domain antibody, and a bivalent domain antibody. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody binds. In certain embodiments, an antigen-binding fragment may comprise one or more CDRs from a particular parent antibody.

[00132] “Fab” with regard to an antibody refers to a monovalent antigen-binding fragment of the antibody consisting of a single light chain (both variable and constant regions) bound to the variable region and first constant region of a single heavy chain by a disulfide bond. Fab can be obtained by papain digestion of an antibody at the residues proximal to the N-terminus of the disulfide bond between the heavy chains of the hinge region.

[00133] “Fab'” refers to a Fab fragment that includes a portion of the hinge region, which can be obtained by pepsin digestion of an antibody at the residues proximal to the C-terminus of the disulfide bond between the heavy chains of the hinge region and thus is different from Fab in a small number of residues (including one or more cysteines) in the hinge region.

[00134] “F(ab')₂” refers to a dimer of Fab' that comprises two light chains and part of two heavy chains.

[00135] “Fv” with regard to an antibody refers to the smallest fragment of the antibody to bear the complete antigen binding site. A Fv fragment consists of the variable region of a single light chain bound to the variable region of a single heavy chain. A “dsFv” refers to a disulfide-

stabilized Fv fragment that the linkage between the variable region of a single light chain and the variable region of a single heavy chain is a disulfide bond.

[00136] “Single-chain Fv antibody” or “scFv” refers to an engineered antibody consisting of a light chain variable region and a heavy chain variable region connected to one another directly or via a peptide linker sequence (Huston JS *et al. Proc Natl Acad Sci USA*, 85:5879(1988)). A “scFv dimer” refers to a single chain comprising two heavy chain variable regions and two light chain variable regions with a linker. In certain embodiments, an “scFv dimer” is a bivalent diabody or bivalent ScFv (BsFv) comprising V_H-V_L (linked by a peptide linker) dimerized with another V_H-V_L moiety such that V_H's of one moiety coordinate with the V_L's of the other moiety and form two binding sites which can target the same antigens (or epitopes) or different antigens (or epitopes). In other embodiments, a “scFv dimer” is a bispecific diabody comprising V_{H1}-V_{L2} (linked by a peptide linker) associated with V_{L1}-V_{H2} (also linked by a peptide linker) such that V_{H1} and V_{L1} coordinate and V_{H2} and V_{L2} coordinate and each coordinated pair has a different antigen specificity.

[00137] “Single-chain Fv-Fc antibody” or “scFv-Fc” refers to an engineered antibody consisting of a scFv connected to the Fc region of an antibody.

[00138] “Camelized single domain antibody,” “heavy chain antibody,” “nanobody” or “HCAb” refers to an antibody that contains two V_H domains and no light chains (Riechmann L. and Muyldermans S., *J Immunol Methods*. Dec 10;231(1-2):25-38 (1999); Muyldermans S., *J Biotechnol*. Jun;74(4):277-302 (2001); WO94/04678; WO94/25591; U.S. Patent No. 6,005,079). Heavy chain antibodies were originally obtained from *Camelidae* (camels, dromedaries, and llamas). Although devoid of light chains, camelized antibodies have an authentic antigen-binding repertoire (Hamers-Casterman C. *et al., Nature*. Jun 3;363(6428):446-8 (1993); Nguyen VK. *et al.* “Heavy-chain antibodies in Camelidae; a case of evolutionary innovation,” *Immunogenetics*. Apr;54(1):39-47 (2002); Nguyen VK. *et al. Immunology*. May;109(1):93-101 (2003)). The variable domain of a heavy chain antibody (VHH domain) represents the smallest known antigen-binding unit generated by adaptive immune responses (Koch-Nolte F. *et al., FASEB J*. Nov;21(13):3490-8. Epub 2007 Jun 15 (2007)). “Diabodies” include small antibody fragments with two antigen-binding sites, wherein the fragments comprise a V_H domain connected to a V_L domain in a single polypeptide chain (V_H-V_L or V_L-V_H) (see, *e.g.*, Holliger P. *et al., Proc Natl Acad Sci U S A*. Jul 15;90(14):6444-8 (1993); EP404097; WO93/11161). The two domains on the same chain cannot be paired, because the linker is too short, thus, the domains are forced to pair with the complementary domains of another chain, thereby creating two antigen-binding sites. The antigen-binding sites may target the same or different antigens (or epitopes).

[00139] A “domain antibody” refers to an antibody fragment containing only the variable region of a heavy chain or the variable region of a light chain. In certain embodiments, two or more V_H domains are covalently joined with a peptide linker to form a bivalent or multivalent domain antibody. The two V_H domains of a bivalent domain antibody may target the same or different antigens.

[00140] In certain embodiments, a “(dsFv)₂” comprises three peptide chains: two V_H moieties linked by a peptide linker and bound by disulfide bridges to two V_L moieties.

[00141] In certain embodiments, a “bispecific ds diabody” comprises V_{H1} - V_{L2} (linked by a peptide linker) bound to V_{L1} - V_{H2} (also linked by a peptide linker) via a disulfide bridge between V_{H1} and V_{L1} .

[00142] In certain embodiments, a “bispecific dsFv” or “dsFv-dsFv” comprises three peptide chains: a V_{H1} - V_{H2} moiety wherein the heavy chains are bound by a peptide linker (*e.g.*, a long flexible linker) and paired via disulfide bridges to V_{L1} and V_{L2} moieties, respectively. Each disulfide paired heavy and light chain has a different antigen specificity.

[00143] As used herein, the term “complex” refers to an aggregate or assembly formed by more than 1 (*e.g.*, 2, 3, 4 or more) proteins. Preferably, a complex has the function that a single protein within the complex does not have. In the complex, the proteins that form the complex may or may not contact with each other.

[00144] As used herein, the term “chimeric”, when used in connection with a protein (*e.g.*, an adaptor protein), refers to a part of the protein is from one species, and the other part of the protein is from another species.

[00145] As used herein, the term “functional variant” refers to a polypeptide/protein that has at least 70% sequence identity to the parent polypeptide/protein yet retains the function of the parent polypeptide/protein. The differences between the variant and the parent polypeptide/protein may be one or more amino acid residues. For example, the functional variant may have substituent, addition, deletion, insertion or truncation of one or more amino acid residue in the parent polypeptide/protein.

[00146] “Percent (%) sequence identity” with respect to amino acid sequence (or nucleic acid sequence) is defined as the percentage of amino acid (or nucleic acid) residues in a candidate sequence that are identical to the amino acid (or nucleic acid) residues in a reference sequence, after aligning the sequences and, if necessary, introducing gaps, to achieve the maximum correspondence.

Alignment for purposes of determining percent amino acid (or nucleic acid) sequence identity can be achieved, for example, using publicly available tools such as BLASTN, BLASTp (available on the website of U.S. National Center for Biotechnology Information (NCBI), see also, Altschul S.F. et al, *J. Mol. Biol.*, 215:403–410 (1990); Stephen F. et al, *Nucleic Acids Res.*, 25:3389–3402 (1997)), ClustalW2 (available on the website of European Bioinformatics Institute, see also, Higgins D.G. et al, *Methods in Enzymology*, 266:383–402 (1996); Larkin M.A. et al, *Bioinformatics* (Oxford, England), 23(21): 2947–8 (2007)), and ALIGN or Megalign (DNASTAR) software. Those skilled in the art may use the default parameters provided by the tool, or may customize the parameters as appropriate for the alignment, such as for example, by selecting a suitable algorithm. In certain embodiments, the non-identical residue positions may differ by conservative amino acid substitutions. A “conservative amino acid substitution” is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, e.g., Pearson (1994) *Methods Mol. Biol.* 24: 307–331, which is herein incorporated by reference.

[00147] The term “operably link” or “operably linked” refers to a juxtaposition, with or without a spacer or linker, of two or more biological sequences (e.g., polynucleotide sequences or amino acid sequences) of interest in such a way that they are in a relationship permitting them to function in an intended manner. When used with respect to proteins (e.g., protein degradation element, chimeric adaptor protein), it is intended to mean that the protein sequences are linked in such a way that permits the linked product to have the intended biological function. The term may also be used with respect to polynucleotides. For instance, when a polynucleotide encoding a protein is operably linked to a regulatory sequence (e.g., promoter, enhancer, silencer sequence, etc.), it is intended to mean that the polynucleotide sequences are linked in such a way that permits regulated expression of the protein from the polynucleotide.

[00148] As used herein, the term “activation” refers to the state of T cells that have been sufficiently stimulated to induce detectable cell proliferation. Activation can also be associated with the production of induced cytokines and detectable effector functions. The term “activated T cells” specifically refers to T cells that are undergoing cell division.

[00149] As used herein, the term “chimeric natural killer receptor”, used interchangeably with the term “CNK receptor” refers to a protein complex that comprises a chimeric NK activation receptor element and a chimeric NK signaling adaptor element, which are coupled to allow signal transduction.

[00150] As used herein, the term “CNK T cell” refers to a T cell expressing the CNK receptor.

[00151] As used herein, the term “chimeric natural killer receptor-Universal T cell”, used interchangeably with the term “CNK-UT”, refers to a T cell that expresses a CNK receptor and be genetically modified to knockout TCR and HLA which allows to generate CNK-T cells from allogeneic healthy donors.

[00152] As used herein, the term “UT” refers to T cells modified (e.g., an immune cell) to be deficient in a molecule that confers GVHD and immunogenicity of the cell (e.g., TCR, MHC class I protein, MHC class II protein or MHC class II transactivator).

[00153] As used herein, the term “therapeutically effective amount” refers to an amount of a cell, composition, formulation or any material as described here effective to achieve a desirable biological result. Such results may include, without limitation, elimination of target cells, such as target T cells associated with diseases (e.g., autoimmune diseases).

[00154] The term “subject” or “individual” or “animal” or “patient” as used herein refers to human or non-human animal, including a mammal or a primate, in need of diagnosis, prognosis, amelioration, prevention and/or treatment of a disease or disorder. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, swine, cows, bears, and so on.

[00155] “Treating” or “treatment” of a condition as used herein includes alleviating a condition, slowing the onset or rate of development of a condition, reducing the risk of developing a condition, preventing or delaying the development of symptoms associated with a condition, reducing or ending symptoms associated with a condition, generating a complete or partial regression of a condition, curing a condition, or some combination thereof.

[00156] The term “vector” as used herein refers to a vehicle into which a polynucleotide encoding a protein may be operably inserted so as to bring about the expression of that protein. A vector may be used to transform, transduce, or transfect a host cell so as to bring about expression of the genetic element it carries within the host cell. Examples of vectors include plasmids, phagemids,

cosmids, artificial chromosomes such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or P1-derived artificial chromosome (PAC), bacteriophages such as lambda phage or M13 phage, and animal viruses. Categories of animal viruses used as vectors include retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (*e.g.*, herpes simplex virus), poxvirus, baculovirus, papillomavirus, and papovavirus (*e.g.*, SV40). A vector may contain a variety of elements for controlling expression, including promoter sequences, transcription initiation sequences, enhancer sequences, selectable elements, and reporter genes. In addition, the vector may contain an origin of replication. A vector may also include materials to aid in its entry into the cell, including but not limited to a viral particle, a liposome, or a protein coating. A vector can be an expression vector or a cloning vector. The present disclosure provides vectors (*e.g.*, expression vectors) containing the nucleic acid sequence provided herein encoding the fusion protein, at least one promoter (*e.g.*, SV40, CMV, EF-1 α) operably linked to the nucleic acid sequence, and at least one selection marker. Examples of vectors include, but are not limited to, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (*e.g.*, herpes simplex virus), poxvirus, baculovirus, papillomavirus, papovavirus (*e.g.*, SV40), lambda phage, and M13 phage, plasmid pcDNA3.3, pMD18-T, pOptivec, pCMV, pEGFP, pIRES, pQD-Hyg-GSeu, pALTER, pBAD, pcDNA, pCal, pL, pET, pGEMEX, pGEX, pCI, pEGFT, pSV2, pFUSE, pVITRO, pVIVO, pMAL, pMONO, pSELECT, pUNO, pDUO, Psg5L, pBABE, pWPXL, pBI, p15TV-L, pPro18, pTD, pRS10, pLexA, pACT2.2, pCMV-SCRIPT.RTM., pCDM8, pCDNA1.1/amp, pcDNA3.1, pRc/RSV, PCR 2.1, pEF-1, pFB, pSG5, pXT1, pCDEF3, pSVSPORT, pEF-Bos etc.

[00157] The phrase “host cell” as used herein refers to a cell into which an exogenous polynucleotide and/or a vector has been introduced.

[00158] **I. Overview**

[00159] The present disclosure is at least partially based on the surprising discovery that a modified cell (*e.g.*, mammalian immune cell) or a population thereof expressing a surface molecule, when activated, is capable of being induced to express one or more induced NK receptor ligand (iNKRL) that is capable of binding to the surface molecule. This may lead to self-killing or fratricidal effect of the modified cell (*e.g.*, mammalian immune cell) or a population thereof, and consequently poor therapeutic efficacy of the modified cell (*e.g.*, mammalian immune cell) or a population thereof, especially when used for treating diseases associated with target cells expressing target NK receptor ligand (tNKRL) (*e.g.*, auto-reactive lymphocytes) in a subject.

[00160] Accordingly, to improve the therapeutic efficacy of cell therapies, the present disclosure provides a modified cell (e.g., mammalian immune cell) or a population thereof, designed in a manner to substantially eliminate the self-killing or fratricidal effect of the modified cell (e.g., mammalian immune cell) or a population thereof. The present disclosure also provides methods of substantially eliminating the self-killing or fratricidal effect and/or improving the therapeutic efficacy of the modified cell (e.g., mammalian immune cell) or a population thereof, by substantially suppressing the expression level of or directly degradation of the one or more iNKRL in the modified cell (e.g., mammalian immune cell) or a population thereof. The present disclosure further provides a method of treating a disease associated with target cells expressing target NK receptor ligand (tNKRL) (e.g., auto-reactive lymphocytes) (such as autoimmune diseases) in a subject, comprising administering to the subject a therapeutically effective amount of the modified cell (e.g., mammalian immune cell) or a population thereof provided herein.

[00161] **II. Modified Cells**

[00162] In one aspect, the present disclosure provides a modified cell (e.g., mammalian immune cell) or a population thereof, comprising a surface molecule and a protection element. In certain embodiments, the surface molecule is expressed on the surface of the modified cell (e.g., immune cell), and is capable of binding to one or more target NK receptor ligand (tNKRL) expressed on a target cell thereby activating the modified cell (e.g., immune cell) to exert cytotoxic effect on the target cell. In certain embodiments, the modified cell (e.g., immune cell), when activated, is capable of being induced to express one or more induced NK receptor ligand (iNKRL) that is capable of binding to the surface molecule. In certain embodiments, the protection element substantially suppresses expression of or degradation of the one or more iNKRL on the modified cell (e.g., immune cell) surface following activation of the modified cell (e.g., immune cell), thereby preventing the activated modified cell (e.g., immune cell) from becoming a target cell.

[00163] In certain embodiments, at least one of the tNKRL is identical to at least one of the iNKRL, or wherein the surface molecule cross-reacts with at least one of the tNKRL and at least one of the iNKRL. In certain embodiments, the one or more tNKRL is selected from the group consisting of NKG2D ligands, NCR ligands, KIR ligands, CD226 ligands, TIGIT ligands, CD96 ligands, or any combination thereof. In certain embodiments, the one or more tNKRL comprises one or more NKG2D ligand. In certain embodiments, the NKG2D ligand is selected from the group consisting of MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6. In certain embodiments, the one or more iNKRL is selected from the group consisting of: NKG2D ligands, NCR ligands, KIR ligands, DNAM1 ligands, CD226 ligands, TIGIT ligands, CD96 ligands, or any

combination thereof. In certain embodiments, the one or more induced NK receptor ligand comprises one or more NKG2D ligand. In certain embodiments, the NKG2D ligand is selected from the group consisting of: MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6. In certain embodiments, the at least one of the tNKRL is identical to at least one of the iNKRL and comprises MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6.

[00164] In certain embodiments, the expression of the one or more iNKRL on the immune cell surface is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%) relative to expression of the one or more iNKRL on a reference immune cell surface without the protection element.

[00165] In certain embodiments, the expression of the one or more iNKRL on the modified cell (e.g., immune cell) surface is reduced such that the binding of the one or more iNKRL to the surface molecule is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%).

[00166] In certain embodiments, the expression of the one or more iNKRL is detectable on no more than 50% (e.g. at least 40%, at least 30%, at least 20%, or at least 10%) of the population of the immune cell following binding of the surface molecule to the one or more tNKRL.

[00167] In certain embodiments, the one or more iNKRL comprises MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6 and the expression level of the MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6 on the immune cell surface is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%).

[00168] In certain embodiments, the one or more iNKRL comprises MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP5 and ULBP6 and the expression level of each of the MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP5 and ULBP6 on the immune cell surface is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%).

[00169] The expression of the iNKRL can be determined using methods known in the art, for example flow cytometry, western blot, or qPCR.

[00170] In certain embodiments, the modified cell (e.g., immune cell) has substantially reduced activation-induced cell death of the modified cell (e.g., immune cell). the reduced activation-induced cell death of the immune cell can be determined by reduced apoptosis detected by Annexin

V/7-AAD. In brief, a cytometric method using Annexin V and 7-AAD can be used to discriminate apoptotic from necrotic cells. 7-amino-actinomycin D (7-AAD) can be excited by the 488nm argon laser line, and is separated from the emissions of FITC and PE, and thus allow characterization of necrotic (7-AAD positive, annexin V negative, and FITC positive cells), apoptotic (7-AAD negative, annexin V negative and FITC positive cells) and viable cells (7-AAD negative, annexin V negative and FITC negative cells) in a subset of phycoerythrin (PE) positive cells. For more details, please see, Herault, O. et al, *British Journal of Haematology*, 1999, **104**, 530–537.

[00171] In certain embodiments, the immune cell is capable of proliferate upon activation (e.g., having an increased proliferation rate as compared to a modified mammalian immune cell without the protection element).

[00172] A. Protection Element

[00173] In certain embodiments, the protection element is capable of silencing expression of the one or more iNKRL or is capable of inducing intracellular retention and/or degradation of the one or more iNKRL.

[00174] In certain embodiments, the protection element comprises a small RNA (e.g. an antisense RNA, an shRNA, an siRNA or miRNA or guide RNA) that targets mRNAs of the one or more iNKRL. The small RNA can also be a vector expressing the above-mentioned small RNA, for example, an antisense RNA, an siRNA or miRNA or guide RNA.

[00175] The small RNA mentioned above generally has a length from 15 to 30bp. RNA interference (RNAi) triggers with larger (>21bp) RNA duplexes interact with the RNAi pathway enzyme Dicer for cleavage and handoff to the RNA-induced silencing complex (RISC) loading complex with an Argonaute (Ago1-Ago4) protein. Shorter (<21bp) siRNAs and analogues can bypass Dicer cleavage and enter the RISC via interactions mediated by the TAR RNA-binding protein (TRBP). The mature RISC can regulate gene expression by inhibiting mRNA translation, inducing mRNA sequestration in cytoplasmic P-bodies and/or GW-bodies, promoting mRNA degradation and directing transcriptional gene silencing of the target gene loci. Detailed description can be found in, for example, *Ryan et al., The current state and future directions of RNAi-based therapeutics. Nature Reviews Drug Discovery volume 18, pages421–446 (2019).*

[00176] In certain embodiments, the protection element comprises a small RNA, such as HCMV encoded microRNAs (miR-20a, miR-93, miR-UL112, as described in, for example, *Shen J et al., Silencing NKG2D ligand-targeting miRNAs enhances natural killer cell-mediated cytotoxicity in breast cancer. Cell Death Dis. 2017 Apr 6;8(4):e2740; Codo P et al., MicroRNA-mediated down-*

regulation of NKG2D ligands contributes to glioma immune escape. Oncotarget (2014) 5(17):7651–62. 10.18632/oncotarget.2287; and Alves E et al., Manipulating the NKG2D Receptor-Ligand Axis Using CRISPR: Novel Technologies for Improved Host Immunity. Front Immunol. 2021 Aug 12;12:712722.) that are able to modulate NKG2D-ligand expression.

[00177] In certain embodiments, the protection element comprises a genome editing system, such as a CRISPR/Cas system to inactivate the one or more gene encoding the one or more iNKRL. In certain embodiments, the CRISPR/Cas system comprises a Cas9 protein and one or more guide RNA (gRNA). In certain embodiments, the gRNA comprises a targeting region that hybridizes with the target sequence, and an activating region that hybridizes with the targeting region to form a double-stranded RNA duplex of a protein-binding segment, wherein the activating region and the targeting region are covalently linked to one another with intervening nucleotides. Once the targeting region of the gRNA hybridizes with the target DNA, the target DNA-gRNA forms a complex with the Cas9 protein, such that the Cas9 protein cleaves the target DNA molecule (e.g., a gene encoding an iNKRL).

[00178] The CRISPR/Cas system can simultaneously target multiple genomic loci by co-expressing a single Cas9 protein with two or more gRNAs, such that the system can be suited for multiple gene inactivation. In certain embodiments, the CRISPR/Cas system comprises a single Cas9 protein and one or more gRNAs that specifically targets one or more gene encoding one or more iNKRL. Examples of a CRISPR/Cas system used to inhibit gene expression are described in U.S. Publication No. 2014/0068797 and U.S. Patent Nos. 8,771,945 and 8,697,359. Endonucleases other than Cas9 may also be used, such as Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Cse1, Cse2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, T7, FokI, other nucleases known in the art, homologs thereof, or modified versions thereof. Methods of inactivating a target gene in an immune cell (e.g., T cells) using CRISPR/Cas9 technology are described, for example, in US Patent Publication Nos. 2016/0272999, 2017/0204372, and 2017/0119820.

[00179] In certain embodiments, the protection element comprises one or more protein degradation element that is capable of inducing intracellular retention and/or degradation of the one or more iNKRL.

[00180] As used herein, the term “protein degradation element” refers to an element that is capable of inducing protein degradation via any protein degradation pathways, such as endoplasmic

reticulum (ER)-associated degradation (ERAD) pathway, ubiquitin proteasome system (UPS)-associated degradation pathway, ubiquitylation-proteasome pathway, or endosome-lysosome pathway, and autophagic degradation pathway.

[00181] Endoplasmic Reticulum-Associated Protein Degradation (ERAD) is a cellular pathway that targets misfolded proteins within the endoplasmic reticulum (ER) for ubiquitination and subsequent degradation by the proteasome. ERAD is the principal stress management mechanism used to clear both misfolded and normal proteins from the ER for cytosolic proteasomal degradation. The process of ERAD can be divided into three steps: (1) recognition of misfolded or mutated proteins within the ER; (2) retrotranslocation of the recognized misfolded or mutated proteins from the ER back into the cytoplasm: terminally misfolded proteins must be retrotranslocated from the ER to the cytoplasm, where the ubiquitin-proteasome system (UPS) is present; (3) ubiquitin-dependent degradation of the recognized misfolded or mutated proteins by the proteasome (see Annamaria et al., ER-associated degradation: Protein quality control and beyond. *J. Cell Biol.* Vol. 204 No. 6 869–879).

[00182] In certain embodiments, at least one of (or all of) the protein degradation elements comprise an endoplasmic reticulum (ER)-retention domain and one or more iNKRL binding domain capable of binding to the one or more iNKRL.

[00183] In certain embodiments, the iNKRL binding domain comprises an antibody domain (e.g. scFv) capable of binding to the one or more iNKRL. In certain embodiments, the one or more iNKRL comprises one or more NKG2D ligands. In certain embodiments, the one or more NKG2D ligands are selected from the group consisting of MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

[00184] In certain embodiments, the iNKRL binding domain is derived from a virus protein capable of binding to the one or more iNKRL. In certain embodiments, the iNKRL binding domain is derived from an ER-retention virus protein.

[00185] In certain embodiments, the iNKRL binding domain is derived from human receptors capable of binding to the one or more iNKRL. In certain embodiments, the iNKRL binding domain is derived from NK receptors.

[00186] In certain embodiments, the protein degradation element further comprises an ER-retention transmembrane domain, wherein the ER-retention transmembrane domain has one end operably linked to the iNKRL binding domain and the other end operably linked to the ER retention domain.

[00187] In certain embodiments, the ER-retention domain and/or the ER-retention transmembrane domain is derived from a human ER-retention protein.

[00188] In certain embodiments, the ER-retention domain and/or the ER-retention transmembrane domain is derived from ER-retention virus protein.

[00189] In certain embodiments, the ER-retention domain and/or the ER-retention transmembrane domain is derived from a recombinant ER-retention protein.

[00190] As used herein, the term “human ER-retention protein” refers to a protein that involves in the ERAD mechanism or pathway. The term “ER-retention domain”, as used herein, refers to a moiety or domain that can bind and/or exploit the human ER-retention protein, such as the transmembrane domain (or a functional variant thereof) and the cytoplasmic domain (or a functional variant thereof) of an ER-retention virus protein. The cytoplasmic domain (or a functional variant thereof) of a human ER-retention protein is also called “ER-retention domain”

[00191] As used herein, the term “ER-retention virus protein” refers to a protein that involves in the ERAD mechanism or pathway. The term “ER-retention domain”, as used herein, refers to a moiety or domain that can bind and/or exploit the ER-retention virus protein, such as the transmembrane domain (or a functional variant thereof) and the cytoplasmic domain (or a functional variant thereof) of an ER-retention virus protein. The cytoplasmic domain (or a functional variant thereof) of an ER-retention virus protein is also called “ER-retention domain”.

[00192] As used herein, the term “recombinant ER-retention protein” refers to a protein that involves in the ERAD mechanism or pathway. The term “ER-retention domain”, as used herein, refers to a moiety or recombinant domain that exploit the human or viral ER-retention protein, such as the transmembrane domain (or a functional variant thereof) and the cytoplasmic domain (or a functional variant thereof). The cytoplasmic domain (or a functional variant thereof) of a recombinant ER-retention protein is also called “ER-retention domain”.

[00193] In certain embodiments, the human ER-retention protein can be the ER resident protein of components of the ERAD machinery. These proteins work together to recognize and target misfolded or unwanted proteins for degradation, eg. Hrd1 and Derlin-1.

[00194] In certain embodiments, the ER-retention virus protein can be the ER resident protein of any virus that can hijack the ERAD mechanism and promote ubiquitination and proteasome-mediated degradation of target proteins.

[00195] In certain embodiments, the ER-retention virus protein is selected from the group consisting of: HCMV protein US2, HCMV protein US11, HCMV protein US18, HCMV protein US20, HCMV protein US3, HCMV protein US10, HCMV protein US6, HSV ICP47, CPXV12, BHV UL49.5, EBV BNFL2a, HCMV protein UL16, HCMV protein UL141, HCMV protein UL142, HIV Nef, HIV Vpu, HHV-7 U21, HHV-8 KK3, HHV-8 KK5, MHV-68 MK3, HTLV-1 p12, Cowpox Virusprotein CPXV203 and Ad E3/19K.

[00196] In certain embodiments, the virus protein capable of binding to the one or more NK receptor ligand is derived from a virus selected from the group consisting of: adenovirus (Ad) (e.g., human Ad), cytomegalovirus (CMV) (human CMV, i.e., HCMV) and Kaposi's sarcoma-associated herpesvirus (KSHV).

[00197] In certain embodiments, the ER-retention domain, and the ER-retention transmembrane domain are both derived from the same ER-retention virus protein.

[00198] In certain embodiments, the ER-retention domain, and the ER-retention transmembrane domain are both derived from the different ER-retention virus protein.

[00199] In certain embodiments, at least one of the ER-retention domain, the ER-retention transmembrane domain and the binding domain, and the other one of the ER-retention domain, the ER-retention transmembrane domain and the binding domain are derived from different human ERAD proteins or different virus proteins.

[00200] In certain embodiments, the ER-retention domain and the binding domain are both derived from the same human ERAD proteins or the same virus protein or fragment thereof.

[00201] In certain embodiments, the ER-retention domain, the ER-retention transmembrane domain and the binding domain are all derived from the same virus protein or fragment thereof.

[00202] In certain embodiments, the same virus protein is selected from the group consisting of HCMV protein UL16, HCMV protein UL141, HCMV protein UL142, HCMV protein US18, HCMV protein US20, HHV-7 U21 and Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), KSHV K5,

[00203] In certain embodiments, the protein degradation element comprises a virus protein selected from the group consisting of HCMV protein UL16, HCMV protein UL141, HCMV protein UL142, HCMV protein US18, HCMV protein US20, KSHV K5 and Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), an analog thereof, or a derivative thereof.

[00204] CMV UL16 binds to MICB and ULBPs (David Cosman et al., 2001); UL141 could promotes efficient downregulation of the DNAM1 ligand CD155 and CD112 and exhibit potent NK cell evasion function (Tomasec P. et al., 2005; Prod'homme V et al.,2010). Moreover, UL141 binding promotes intracellular retention of the TRAIL Death Receptors, thus protecting virus infected cells from TRAIL-dependent NK cell-mediated killing. (Smith W et al.,2013) UL142 could recognize and intracellular sequestration of full-length MICA alleles in endoplasmic reticulum (ER) and cis-Golgi apparatus (Omodele Ashiru et al., 2009). HCMV targets MICA via US18 and US20 for lysosomal degradation (Ceri A. Fielding et al., 2014). HHV-7 U21 can associate with the NKG2D ligand MICA/MICB and ULBP1 and redirect it to lysosomes for degradation (Schneider CL, Hudson AW. PLoS Pathog. 2011). HHV-8(KSHV) K5 ubiquitin ligase promotes the degradation of MICA and MICB (Thomas M et al., PNAS, 2008). The E3 19K protein of adenoviruses has been found to directly bind to MHC class I molecules and MICA/MICB in the endoplasmic reticulum (ER), resulting in the retention and downregulation of MHC class I molecules (Brian P. McSharry et al., 2008);

[00205] In certain embodiments, the one or more protein degradation elements comprise a combination of virus proteins.

[00206] In certain embodiments, the combination of virus proteins is selected from the group consisting of:

- a) HCMV protein UL16 and HCMV protein UL142;
- b) HCMV protein UL16 and HCMV protein US18;
- c) HCMV protein UL16 and HCMV protein US20;
- d) protein UL142 and HCMV protein US18;
- e) protein UL142 and HCMV protein US20;
- f) HCMV protein US18 and HCMV protein US20;
- g) Ad E3/19K and HCMV protein UL16;
- h) Ad E3/19K and HCMV protein UL142;
- i) Ad E3/19K and HCMV protein US18;
- j) Ad E3/19K and HCMV protein US20;
- k) HCMV protein US20, HCMV protein US18 and HCMV protein UL142;
- l) HCMV protein US20, HCMV protein UL16 and HCMV protein US18;
- m) HCMV protein US20, HCMV protein UL16 and HCMV protein US20;
- n) HCMV protein US20, HCMV protein UL16 and HCMV protein UL142;

- o) HCMV protein US20, HCMV protein US20 and HCMV protein US18;
- p) Ad E3/19K, HCMV protein UL142 and HCMV protein US20;
- q) Ad E3/19K, HCMV protein US18 and HCMV protein UL142;
- r) Ad E3/19K, HCMV protein UL16 and HCMV protein US20;
- s) Ad E3/19K, HCMV protein UL16 and HCMV protein US18;
- t) Ad E3/19K, HCMV protein UL16 and HCMV protein UL142;
- u) Ad E3/19K, HCMV protein US18, HCMV protein UL142 and HCMV protein US20;
- v) Ad E3/19K, HCMV protein UL16, HCMV protein US20 and HCMV protein UL142;
- w) Ad E3/19K, HCMV protein UL16, HCMV protein US18 and HCMV protein UL142;
- x) Ad E3/19K, HCMV protein UL16, HCMV protein US18 and HCMV protein US20;
- y) HCMV protein UL16, HCMV protein US18, HCMV protein US20 and HCMV protein UL142;
and
- z) Ad E3/19K, HCMV protein UL16, HCMV protein US18, HCMV protein US20 and HCMV protein UL142.

[00207] In certain embodiments, the combination of virus proteins comprises a HCMV protein UL16 and another ER-retention virus protein. In certain embodiments, the other ER-retention virus protein is selected from the group consisting of HCMV protein UL141, HCMV protein UL142, HCMV protein US18, HCMV protein US20, HHV-7 U21, HHV-8 K5 and Ad E3/19K (e.g., Ad2 E3/19K, Ad3 E3/19K, Ad5 E3/19K, Ad6 E3/19K, Ad11 E3/19K, Ad35 E3/19K).

[00208] The amino acid sequences of the ER-retention virus proteins are provided in Table 2. Also these sequences are available from the published PCT application WO2022161502A1, which is incorporated herein to its entirety.

[00209] In certain embodiments, the one or more protein degradation elements further comprise a protein degradation pathway (PDP) binding domain capable of binding to a member in a protein degradation pathway.

[00210] In certain embodiments, the PDP binding domain is operably linked to the ER-retention domain.

[00211] In certain embodiments, the protein degradation pathway is ubiquitylation-proteosome pathway, endosome-lysosome pathway, or autophagic degradation pathway.

[00212] In certain embodiments, the member in the ubiquitylation-proteosome pathway comprises E1 ubiquitin activating enzyme, E2 ubiquitin conjugating enzyme, or E3 ubiquitin ligase.

[00213] Examples of E1 ubiquitin-activating enzymes include UBA1, UBA2, UBA3, UBA5, UBA6, UBA7, ATG7, NAE1, and SAE1.

[00214] Examples of E2 ubiquitin-conjugating enzymes include hCdc34, Ubc-Uev1A, UBE2A, UBE2B, UBE2C, UBE2D1, UBE2D2, UBE2D3, UBE2D4, UBE2E1, UBE2E2, UBE2E3, UBE2F, UBE2G1, UBE2G2, UBE2H, UBE2I, UBE2J1, UBE2J2, UBE2K, UBE2L3, UBE2L6, UBE2M, UBE2N, UBE2O, UBE2Q1, UBE2Q2, UBE2R1 (CDC34), UBE2R2, UBE2S, UBE2T, UBE2U, UBE2V1, UBE2V2, UBE2W, UBE2Z, ATG3, BIRC6, and UFC1.

[00215] Examples of E3 ubiquitin ligases include von Hippel-Lindau (VHL), Cereblon (CRBN), inhibitor of apoptosis protein (IAP), Kelch-like ECH-associated protein 1 (Keap1), RNF4, RNF114, MDM2, LUBAC, FBW7, Met30, HECT, SKP2, beta TRCP1, HUWEI, TRAF6, SMURF1, and E6AP. In certain embodiments, examples of E3 ubiquitin ligases include E3A, MDM2, Anaphase-promoting complex (APC), UBR5 (EDD1), SOCS/BC-box/eloBC/CUL5/RING, LNXp80, CBX4, CBLL1, HACE1, HECTD1, HECTD2, HECTD3, HECTD4, HECW1, HECW2, HERC1, HERC2, HERC3, HERC4, HERC5, HERC6, HUWE1, ITCH, NEDD4, NEDD4L, PPIL2, PRPF19, PIAS1, PIAS2, PIAS3, PIAS4, RANBP2, RNF4, RBX1, SMURF1, SMURF2, STUB1, TOPORS, TRIP12, UBE3A, UBE3B, UBE3C, UBE3D, UBE4A, UBE4B, UBOX5, UBR5, VHL, WWP1, WWP2, Parkin, and MKRN1.

[00216] In certain embodiments, the member in the endosome-lysosome pathway comprises AP-1, AP-2, AP-3, endosome, lysosome, HOPS, ESCRT, GASP, BLOC-1, ESCRT, Retromer, ESCRT, sortingnexin, Dapper2, SNX4, Pincher, Rap1-PDZ-GEF1, clathrin, or C3G/CrkL/Shp2/Gab2.

[00217] In certain embodiments, the member in the autophagic degradation pathway comprises chaperone-mediated autophagy (CMA) USP10, G3BP1, ULK1, ATG16L1, TRIM16, FBXO27VDAC, RHOT1, MFN1/2, BNIP3L, FUNDC1, BNIP3, AMBRA1, BCL2LI3, FKBP8, CHDH, DISC1, PHB2, Cardiolipin, SEC62, RTN3, PEX5, PEX14, ABCD3, or NUFIP1.

[00218] B. Surface Molecule

[00219] In certain embodiments, the surface molecule comprises: a) a tNKRL-binding extracellular domain capable of binding to the one or more target NK receptor ligand, and b) a surface molecule (SM) intracellular domain capable of activating the immune cell following binding of the tNKRL-binding extracellular domain to the one or more target NK receptor ligand, and wherein the tNKRL-binding extracellular domain is associated with the SM intracellular domain to allow

transduction of binding signal from the tNKRL-binding extracellular domain to the SM intracellular domain.

[00220] In certain embodiments, the tNKRL-binding extracellular domain comprises an antibody domain that is capable of binding to the one or more target NK receptor ligand.

[00221] In certain embodiments, the tNKRL-binding extracellular domain comprises an NK receptor extracellular domain that is capable of binding to the one or more target NK receptor ligand.

[00222] In certain embodiments, the tNKRL-binding extracellular domain comprises an NKG2D extracellular domain.

[00223] In certain embodiments, the SM intracellular domain comprises a cytoplasmic domain of a protein selected from the group consisting of: 4-1BB (CD137), CD2, CD3, CD35, CD25, CD27, CD28, CD30, CD40, CD79A, CD79B, CARD11, DAP10, DAP12, Fc receptor, Fyn, LIGHT, LT β R, HVEM, ICOS, Lck, LAG3, LAT, LRP, NOTCH1, NOTCH2, NOTCH3, NOTCH4, OX40 (CD134), ROR2, Ryk, SLAMF1, Slp76, pTa, TCR α , vTCRP, TRIM, Zap70, PTCH2, IL7 receptor, IL15 receptor and GITR.

[00224] In certain embodiments, the SM intracellular domain comprises a cytoplasmic domain of CD3 (e.g., CD3 zeta).

[00225] In certain embodiments, the SM intracellular domain comprises the amino acid sequence of SEQ ID NO: 5.

[00226] In certain embodiments, the receptor further comprises a SM transmembrane domain, wherein the SM transmembrane domain has one end operably linked to the tNKRL-binding extracellular domain and the other end operably linked to the SM intracellular domain.

[00227] In certain embodiments, the SM transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of CD3, CD4, CD8, CD28, and CD137.

[00228] In certain embodiments, the surface molecule comprises a tNKRL-binding chimeric antigen receptor (CAR).

[00229] In certain embodiments, the surface molecule comprises a CNK receptor. In certain embodiments, the CNK receptor comprises a protein complex comprising a first polypeptide comprising the tNKRL-binding extracellular domain operably linked to a first CNK transmembrane domain, and a second polypeptide comprising the CNK intracellular domain operably linked to a

second CNK transmembrane domain, wherein the first CNK transmembrane domain and the second CNK transmembrane domain associate with each other to form the protein complex.

[00230] In certain embodiments, the first CNK transmembrane domain comprises a NK receptor transmembrane domain, optionally an NKG2D transmembrane domain.

[00231] In certain embodiments, the first CNK transmembrane domain is capable of forming a first dimer.

[00232] In certain embodiments, the first polypeptide comprises a NKG2D protein.

[00233] In certain embodiments, the NKG2D protein comprises the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2.

[00234] In certain embodiments, the second CNK transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of DNAX-Activating Protein of 10 kDa (DAP10) and DNAX-Activating Protein of 12 kDa (DAP12).

[00235] In certain embodiments, the CNK intracellular domain comprises a cytoplasmic domain of a protein selected from the group consisting of: 4-1BB (CD137), CD2, CD3, CD35, CD25, CD27, CD28, CD30, CD40, CD79A, CD79B, CARD11, DAP10, DAP12, Fc receptor, Fyn, LIGHT, LT β R, HVEM, ICOS, Lck, LAG3, LAT, LRP, NOTCH1, NOTCH2, NOTCH3, NOTCH4, OX40 (CD134), ROR2, Ryk, SLAMF1, Slp76, pTa, TCR α , vTCRP, TRIM, Zap70, PTCH2, IL7 receptor, IL15 receptor and GITR.

[00236] In certain embodiments, the CNK intracellular domain comprises a cytoplasmic domain of CD3 (e.g., CD3 zeta).

[00237] In certain embodiments, the second polypeptide comprises a chimeric adaptor protein comprising:

- a) the transmembrane domain of DAP10 operably linked to the intracellular domain of CD3 (e.g., CD3 zeta);
- b) the transmembrane domain of DAP12 operably linked to the intracellular domain of CD3 (e.g., CD3 zeta); or
- c) the transmembrane domain of DAP10 operably linked to the intracellular domain of DAP12.

[00238] In certain embodiments, the chimeric adaptor protein comprises the amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 16.

[00239] In certain embodiments, the second transmembrane domain is capable of forming a second dimer, and wherein the second dimer associates with the first transmembrane domain to form the complex.

[00240] In certain embodiments, the CNK receptor comprises a hexameric protein complex comprising a homodimer of the NKG2D protein and two homodimers of the chimeric adaptor protein.

[00241] In certain embodiments, the genetically modified immune cell comprises a CNK-UT cell.

[00242] In certain embodiments, the immune cell is further modified to be deficient in a) MHC class I protein, or b) MHC class II protein or MHC class II transactivator, or c) both a) and b).

[00243] In some embodiments, the MHC class I protein comprises HLA-A, or HLA-B, HLA-C, B2M, or any combination thereof, optionally, the cell is further deficient in both HLA-A and HLA-B. In certain embodiments, the MHC class II protein comprises HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, HLA-DR, or any combination thereof, optionally, the cell is further deficient in HLA-DR. In certain embodiments, the MHC class II transactivator is CIITA, optionally, the cell is deficient in CIITA. In some embodiments, the cell is further deficient in endogenous TCR, optionally, the cell is further deficient in T cell receptor alpha chain constant region (TRAC), T cell receptor beta constant 1 (TRBC1), T cell receptor beta constant 2 (TRBC2), or any combination thereof.

[00244] Any suitable methods can be used to modify a cell to be deficient in the endogenous a) MHC class I protein, or b) MHC class II protein or MHC class II transactivator, or c) both a) and b), for example, by gene editing, by interfering with expression of the endogenous a) MHC class I protein, or b) MHC class II protein or MHC class II transactivator, or c) both a) and b), or by promoting degradation of endogenous a) MHC class I protein, or b) MHC class II protein or MHC class II transactivator, or c) both a) and b) or its encoding mRNA.

[00245] In certain embodiments, the modified cell (e.g., mammalian immune cell) or a population thereof comprises a UT element. In certain embodiments, the UT element comprises a small RNA (e.g. an siRNA or miRNA or guide RNA) that targets mRNAs of a) MHC class I protein, or b) MHC class II protein or MHC class II transactivator, or c) both a) and b). In certain embodiments,

the UT element comprises a genome editing system, such as a CRISPR/Cas system to inactivate the one or more gene encoding a) MHC class I protein, or b) MHC class II protein or MHC class II transactivator, or c) both a) and b). In certain embodiments, the UT element comprises one or more protein degradation element that is capable of inducing intracellular retention and/or degradation of a) MHC class I protein, or b) MHC class II protein or MHC class II transactivator, or c) both a) and b).

[00246] In certain embodiments, the immune cell is T cell and the immune cell is further modified to be deficient in endogenous TCR.

[00247] C. Other Synthetic Receptors (e.g., CAR, TCR)

[00248] In certain embodiments, the genetically modified immune cell provided herein further expresses a target binding (TB) receptor capable of binding to a target marker.

[00249] In certain embodiments, the target binding (TB) receptor comprises a chimeric antigen receptor (CAR). In certain embodiments, the CAR comprises a target protein binding domain (e.g., extracellular recognition domain targeting the tumor), a transmembrane domain, and an immune receptor activation signaling domain (ITAM, also known as "intracellular signaling domain"). In some embodiments, the CAR may further include a co-stimulatory domain. In certain embodiments, there may be hinges or linkers between the extracellular recognition domain targeting the target marker, the transmembrane domain, and/or the intracellular signaling domain.

[00250] In certain embodiments, the target binding (TB) receptor comprises an engineered T cell receptor (TCR) that is engineered to specifically bind to the target.

[00251] In certain embodiments, the target marker is expressed on the target cell.

[00252] In certain embodiments, the TB receptor comprises a target-marker binding extracellular domain capable of binding to the target marker and a TB intracellular domain capable of activating the immune cell upon binding of the target-marker binding extracellular domain to the target marker. In some embodiments, the extracellular recognition domain of the CAR is selected from antibodies or functional fragments thereof that can specifically recognize tumor-associated antigens, T cell receptors (TCR), or their combinations. The functional fragments of antibodies include Fd, Fv, Fab, Fab', F(ab')₂, Fv(scFv), single-chain antibodies (scFv), or nanobodies, as well as bispecific, trispecific, and tetravalent antibodies.

[00253] In certain embodiments, the TB receptor further comprises a TB transmembrane domain. In some embodiments, the transmembrane domain of the CAR can be derived from any

membrane-bound or transmembrane protein, including but not limited to BAFFR, BLAME (SLAMF8), CD2, CD3 ϵ , CD4, CD5, CD8, CD9, CD11a (CD18, ITGAL, LFA-1), CD11b, CD11c, CD11d, CD16, CD19, CD22, CD27, CD28, CD29, CD33, CD37, CD40, CD45, CD49a, CD49d, CD49f, CD64, CD80, CD84, CD86, CD96 (Tactile), CD100 (SEMA4D), CD103, CD134, CD137 (4-1BB), CD150 (IPO-3, SLAMF1, SLAM), CD154, CD160 (BY55), CD162 (SELPLG), CD226 (DNAM1), CD229 (Ly9), CD244 (2B4, SLAMF4), CD278 (ICOS), CEACAM1, CRTAM, GTR, HYEM (LIGHTR), IA4, IL2R β , IL2R γ , IL7R α , ITGA1, ITGA4, ITGA6, ITGAD, ITGAE, ITGAM, ITGAX, ITGB1, ITGB2, ITGB7, KIR, LTBR, OX40, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), PAG/Cbp, PSGL1, SLAMF6 (NTB-A, Ly108), SLAMF7, T cell receptor α , β , or ζ chains, TNFR2, VLA1, or VLA-6.

[00254] In some embodiments, the transmembrane domain of the CAR is derived from NK cell activation receptor transmembrane domains, DAP10 transmembrane domain, DAP12 transmembrane domain, CD8 transmembrane domain, CD28 transmembrane domain, CD4 transmembrane domain, 4-1BB transmembrane domain, OX40 transmembrane domain, ICOS transmembrane domain, CTLA-4 transmembrane domain, PD-1 transmembrane domain, LAG-3 transmembrane domain, 2B4 transmembrane domain, or BTLA transmembrane domain, as well as combinations thereof.

[00255] In certain embodiments, the TB transmembrane domain has one end operably linked to the target-marker binding extracellular domain and the other end operably linked to the TB intracellular domain.

[00256] In some embodiments, the immune receptor activation signaling domain (ITAM) of the CAR is derived from intracellular activation signaling domains of immune receptors. Preferably, the immune receptors are selected from TCR ζ , CD2, CD3 γ , CD3 δ , CD3 ϵ , CD3 ζ , CD5, CD22, Fc γ R, CD66d, Fc α RI, Fc γ RI, Fc γ RII, Fc γ RIII, Dectin-1, CLEC-1, CD72, CD79A, CD79B, or any combinations thereof. Alternatively, the ITAM of the immune receptor is fused with NK cell signal transducers or their functional variants, and the immune receptor is CD3 ζ .

[00257] In some preferred embodiments, the ITAM of the CAR is derived from intracellular signal domains of immune receptors, including but not limited to CD3 ζ , common Fc γ R (FCER1G), Fc γ RIIa, FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b, CD278 ("ICOS"), Fc ϵ RI CD66d, DAP10, or DAP12.

[00258] In some embodiments, the intracellular signaling domains of the CAR include the intracellular signal domains of NK cell activation receptors and/or co-stimulatory signaling

domains. In some embodiments, the T cell co-stimulatory signaling domains of the CAR are derived from the intracellular signal domains of co-stimulatory molecules. Preferably, the co-stimulatory molecules are selected from MHC class I molecules, TNF receptor proteins, immunoglobulin-like proteins, cytokine receptors, integrins, lymphocyte activation signaling molecules (SLAM proteins), activating NK cell receptors, BTLA, Toll-like receptor ligands, OX40, CD2, CD7, CD16, CD27, CD28, CD30, CD40, CD38, CD35, CD79A, CD79B, CDS, ICAM-1, LFA-1, (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8 α , CD8 β , IL2R β , IL2R γ , IL7R α , ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, NCR, DAP10, DAP12, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, ligands specifically binding to CD83, CARD11, FcRa, FcRp, FcRy, Fyn, HVEM, ICOS, Lck, LAG3, LAT, LRP, NOTCH1, Wnt, OX40, ROR2, Ryk, SLAMF1, Slp76, pTa, TCRa, TCRp, TRIM, ZAP70, PTCH2. More preferably, the co-stimulatory signaling domain of the CAR can be derived from NKG2D intracellular signal domain, DAP10 intracellular signal domain, DAP12 intracellular signal domain, NCR intracellular signal domain, CD28 intracellular signal domain, 4-1BB intracellular signal domain, OX40 intracellular signal domain, ICOS intracellular signal domain, CTLA-4 intracellular signal domain, PD-1 intracellular signal domain, LAG-3 intracellular signal domain, 2B4 intracellular signal domain, BTLA intracellular signal domain, or any combinations thereof.

[00259] In certain embodiments, the target marker is a tumor antigen, an auto-reactive cell antigen, a virus antigen, bacteria antigen, a microbe-infected cell, or damaged and/or aged cells.

[00260] In certain embodiments, the target cell is a tumor cell, an auto-reactive cell, or a virus-infected cell.

[00261] In certain embodiments, the immune cell is a T cell, a Natural Killer (NK) cell, a NKT cell, a B cell, a hematopoietic cell (e.g., bone marrow cell), a thymocyte, a dendritic cell (e.g., a mature dendritic cell), a macrophage cell, a tumor infiltrating lymphocyte, a monocyte, a granulocyte.

[00262] In certain embodiments, the immune cell is a T cell, such as CD4⁺ T cell, CD8⁺ T cell, cytotoxic T cell, terminal effector T cell, memory T cell, naïve T cell, natural killer T cell, gamma-delta T cell, cytokine-induced killer (CIK) T cell, or tumor infiltrating lymphocyte.

[00263] **II. Expression construct and methods of production**

[00264] In another aspect, the present disclosure provides an expression construct encoding a surface molecule described herein and a protection element described herein, wherein the construct can be expressed in an immune cell.

[00265] In some embodiments, the vector is selected from plasmids, nanoplasms, viral vectors, episomes, RNA vectors, or linear or circular DNA (e.g., transposon DNA) or RNA molecules.

[00266] In some embodiments, the viral vector is selected from retroviruses, lentiviral vectors, adenoviruses, adeno-associated virus (AAV) vectors, coronaviruses, negative-sense RNA viruses such as orthomyxoviruses (e.g., influenza virus), rhabdoviruses (e.g., rabies virus and vesicular stomatitis virus), paramyxoviruses (e.g., measles and Sendai viruses), positive-sense RNA viruses such as picornaviruses and togaviruses, and double-stranded DNA viruses, including adenoviruses, herpesviruses (e.g., herpes simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxviruses (e.g., vaccinia virus, variola virus, and canarypox virus), Norwalk virus, reoviruses, yellow fever virus, caliciviruses, hepaciviruses, picobirnaviruses, circoviruses, hepatitis viruses, and virus-like particles (VLPs).

[00267] In some embodiments, the viral vector is a retroviral vector.

[00268] In some embodiments, the retrovirus is selected from avian leukosis-sarcoma virus, mammalian C-type, B-type viruses, D-type viruses, HTLV-BLV complex, lentiviruses, and foamy viruses.

[00269] In some embodiments, the viral vector is a lentiviral vector. In some embodiments, the viral vector is an oncolytic virus vector. Oncolytic virus vectors refer to viral vectors based on oncolytic viruses, which are capable of inserting exogenous nucleic acid sequences.

[00270] In some embodiments, the lentiviral vector is selected from HIV-1, HIV-2, SIV, FIV, BIV, EIAV, CAEV, or maedi-visna lentiviruses.

[00271] In some embodiments, the vector is based on a transposon-based expression vector. Transposons are DNA sequences that can change their position within the genome. In a transposon system, the nucleic acid molecule encoding the chimeric protein construct provided herein has terminal repeat sequences that are recognized by the transposase, which mediates the

transposition. The transposase can be co-delivered as a protein, encoded on the same vector as the chimeric protein construct, or encoded on a separate vector. Non-limiting examples of transposon systems include Sleeping Beauty, PiggyBac, Frog Prince, and Prince Charming.

[00272] In some embodiments, the vector also includes a promoter; preferably, the promoter is EF1 α promoter or CMV promoter.

[00273] In another aspect, the present disclosure provides a method of producing the genetically modified mammalian immune cell described herein, comprising introducing the expression construct described herein to a starting immune cell under conditions to allow expression of the surface molecule on the surface of the immune cell and to allow expression of the protection element in the immune cell.

[00274] Many of the known methods for generating engineered T cells in the field can also be applied to produce engineered cells as described herein. For instance, Zhang et al., "Engineering CAR-T cells" in Biomarker Research (2017) 5:22, describes methods for generating CAR-T cells. The methods provided herein may include one or more of the following steps: obtaining starting cells, culturing (including expanding, optionally activating) the starting cells, and genetically modifying the cells. The starting cells can be stem cells, such as hematopoietic progenitor cells (e.g., T cell progenitors, NK cell progenitors, macrophage progenitors), hematopoietic stem cells (HSCs), CD34⁺ cells, embryonic stem cells, mesenchymal stem cells, or iPSC cells. The starting cells can also be differentiated cells derived from stem cells, such as the immune cells mentioned above.

[00275] The starting cells can be obtained from any source, for example, isolated from the immune cells (e.g., T cells) of a subject (e.g., human subject). In some embodiments, the immune cells are obtained from the subject of interest, such as subjects suspected to have specific diseases or conditions, susceptible subjects to specific diseases or conditions, subjects undergoing specific treatments for certain diseases or conditions, healthy volunteers, or healthy donors. The immune cells can also be sourced from a blood bank. The immune cells for the subject of interest can be autologous or allogeneic. The immune cells can be collected from any location they exist in the subject, including but not limited to blood, cord blood, spleen, thymus, lymph nodes, pleural fluid, splenic tissue, tumors, and bone marrow. Isolated immune cells can be used directly or stored for a period, such as cryopreservation.

[00276] In certain embodiments, the starting immune cells (e.g., T cells) are taken from a healthy donor (e.g. a mammalian donor), and are expanded and activated in vitro. In certain embodiments, the starting mammalian immune cells (e.g., T cells) are modified in vitro to the

genetically modified mammalian immune cell or a population thereof provided herein. The starting immune cells (e.g., T cells) or the genetically modified mammalian immune cell or a population thereof provided herein that are most active against the disease associated with auto-reactive T lymphocytes are cultured in large batches in vitro for about 2 weeks. The in vitro cultured genetically modified mammalian immune cell or a population thereof provided herein will be infused into the patient.

[00277] In certain embodiments, the immune cells are activated and/or expanded. In some embodiments, immune cells are activated and expanded simultaneously, before, and/or after genetic modification. In some embodiments, immune cells are activated and/or expanded ex vivo, in vitro, or in vivo. Methods for activating and expanding immune cells have been described in the art and can be used in the methods described in this paper. For instance, T cells can be activated and expanded by contacting them with agents that provide signals through the stimulatory CD3/TCR complex and co-stimulatory molecules on the surface of T cells. Specifically, T cell populations can be stimulated by contacting them with anti-CD3 antibodies or their antigen-binding fragments or antibodies fixed on the surface, or with protein kinase C activators (e.g., phorbol esters) and calcium ionophores. Co-stimulatory molecules on the T cell surface can be stimulated by using ligands that bind to co-stimulatory molecules. For example, under conditions suitable for stimulating T cell proliferation, T cell populations can be contacted with anti-CD3 antibodies and anti-CD28 antibodies. For stimulating proliferation of CD4⁺ T cells or CD8⁺ T cells, anti-CD3 antibodies and anti-CD28 antibodies can be used. In some embodiments, primary stimulatory signals and co-stimulatory signals for T cells can be provided by different means.

[00278] Genetic modification of cells can be achieved by transducing substantially homogeneous starting cells with nucleic acid molecules encoding the surface molecules and protection elements provided herein. In some embodiments, retroviral vectors (e.g., lentiviral vectors) can be used to introduce nucleic acid molecules provided in this paper into starting cells. For example, the nucleic acid molecules provided herein can be cloned into lentiviral vectors and expressed under the control of their endogenous promoter, lentiviral long terminal repeat sequences, or a promoter specific for the cell type of interest. Common delivery methods for delivering viral vectors include, but are not limited to, electroporation, microinjection, gene gun, and magnetofection.

[00279] Genetic modification of cells can also be achieved by delivering nucleic acid molecules using lipid nanoparticles (LNPs). Other non-viral methods for nucleic acid delivery include the use of calcium phosphate, DEAE-dextran, electroporation, and protoplast fusion for in vitro transfection. Transposases or targeted nucleases (e.g., zinc finger nucleases, meganucleases,

TALE nucleases, CRISPR) can also be used to genetically modify starting cells and obtain engineered cells as described in this disclosure.

[00280] In some embodiments, engineered cells provided herein can be prepared by transfecting nucleic acid molecules encoding the construct provided herein into the starting cells before administration. In some embodiments, engineered cells provided herein can be prepared by transfecting immune cells with nucleic acid molecules encoding the chimeric protein construct, for example, using viral vectors, before administration. Engineered cells provided herein exhibit reduced expression of immunogenic molecules (e.g., TCR, HLA, etc.) and/or immunosuppressive molecules (e.g., PD-1) and/or enhanced expression of tumor-targeting receptors (e.g., CARs, engineered TCRs, CNK receptors) on the cell surface.

[00281] **III. Pharmaceutical composition**

[00282] In another aspect, the present disclosure also provides a pharmaceutical composition comprising the genetically modified mammalian immune cell or the population thereof provided herein and a pharmaceutically acceptable medium. As used herein, the term “pharmaceutical composition” refers to a composition formulated for pharmaceutical use.

[00283] The term “pharmaceutically acceptable” indicates that the designated carrier, vehicle, diluent, excipient(s), and/or salt is generally chemically and/or physically compatible with the other ingredients comprising the formulation, and physiologically compatible with the recipient thereof.

[00284] A “pharmaceutically acceptable medium” refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is bioactivity acceptable and nontoxic to a subject. Pharmaceutical acceptable medium for use in the pharmaceutical compositions disclosed herein may include, for example, pharmaceutically acceptable liquid, gel, or solid carriers, aqueous or nonaqueous vehicles, antimicrobial agents, buffers, antioxidants, isotonic agents, suspending/dispersing agents, sequestering or chelating agents, diluents, adjuvants, excipients, or non-toxic auxiliary substances, or various combinations thereof.

[00285] The pharmaceutical compositions of the present disclosure can be prepared using various techniques known in the art, see, for example, Remington, The Science and Practice of Pharmacy (21st ed. 2005). Briefly, the genetically modified mammalian immune cell or the population thereof is admixed with a suitable medium prior to use or storage. Suitable pharmaceutically acceptable medium generally comprises inert substances that help in: 1) administering the pharmaceutical composition to a subject, 2) processing the pharmaceutical

compositions into deliverable preparations, and/or 3) storing the pharmaceutical composition prior to administration. In certain embodiments, the pharmaceutically acceptable medium comprises agents that can stabilize, optimize or alter the form, consistency, viscosity, pH, pharmacokinetics, and/or solubility of the formulation. Such agents include, without limitation, buffering agents, wetting agents, emulsifying agents, diluents, encapsulating agents, and skin penetration enhancers, for example, saline, buffered saline, dextrose, arginine, sucrose, water, glycerol, ethanol, sorbitol, dextran, sodium carboxymethyl cellulose, and combinations thereof.

[00286] Exemplary pharmaceutically acceptable medium include sugars (e.g., lactose, glucose and sucrose), starches (e.g., corn starch and potato starch), cellulose and derivatives thereof (e.g., sodium carboxymethyl cellulose, methylcellulose, ethyl cellulose, microcrystalline cellulose and cellulose acetate), powdered tragacanth, malt, gelatin, lubricating agents (e.g., magnesium stearate, sodium lauryl sulfate and talc), excipients (e.g., cocoa butter and suppository waxes), oils (e.g., peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil, glycols (e.g., propylene glycol), polyols (e.g., glycerin, sorbitol, mannitol and polyethylene glycol (PEG)), esters (e.g., ethyl oleate and ethyl laurate), agar, buffering agents (e.g., magnesium hydroxide and aluminum hydroxide), alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, pH buffered solutions, polyesters, polycarbonates, polyanhydrides, bulking agents (e.g., polypeptides and amino acids, serum alcohols (e.g., ethanol), (sterile) phosphate-buffered saline, Ringer's solution, dextrose solution and other non-toxic compatible substances used in pharmaceutical formulations.

[00287] Pharmaceutical compositions of the present invention may comprise the genetically modified mammalian immune cell or the population thereof as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present invention are preferably formulated for intravenous administration.

[00288] **IV. Kit**

[00289] In another aspect, the present disclosure also provides a kit comprising the genetically modified mammalian immune cell or the population thereof provided herein. In another

aspect, the present disclosure also provides a kit comprising the expression construct provided herein for use in generating the genetically modified mammalian immune cell or the population thereof.

[00290] In some embodiments, the kits of the present disclosure comprise written instructions for the use of the kit. In certain embodiments, the instructions include at least one of the following: clinical studies, precautions, warnings, and/or references. The instructions can be either printed directly on the container (when present) or provided in the container or with the container as a label applied to the container, or as a separate sheet, pamphlet, card, or folder. Suitable containers include, for example, bottles, syringes, vials, and test tubes. The containers can be formed from a variety of materials such as plastic or glass. In certain embodiments, the container holds the pharmaceutical composition provided herein and have a sterile access port.

[00291] In certain embodiments, the kit further comprises a second container comprising a pharmaceutically acceptable medium as described above. In certain embodiments, the kit further comprises other materials that are commercially desirable or user friendly, such as other diluents, buffers, needles, filters, syringes, and package inserts with instructions for use.

[00292] **V. Method of Treatment**

[00293] In another aspect, the present disclosure provides a method of treating a disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the genetically modified mammalian immune cell or a population thereof provided herein, or the pharmaceutical composition provided herein. In certain embodiments, the disease is associated with target cells expressing tNKRL (e.g., auto-reactive lymphocytes). In certain embodiments, the disease is cancer. In certain embodiments, the disease is autoimmune disease. In certain embodiments, the disease is an immune deficient disease, or an aging associated disease.

[00294] In certain embodiments, the disease associated with auto-reactive lymphocytosis is an autoimmune disease.

[00295] In certain embodiments, the autoimmune disease is organ-specific autoimmune disease or systemic autoimmune disease.

[00296] In certain embodiments, the organ-specific autoimmune disease is selected from the group consisting of chronic lymphocytic thyroiditis, hyperthyroidism, insulin-dependent diabetes mellitus, myasthenia gravis, ulcerative colitis, pernicious anemia with chronic atrophic gastritis, pulmonary hemorrhage nephritic syndrome, pemphigus vulgaris, pemphigoid, primary biliary cirrhosis, multiple sclerosis, acute idiopathic polyneuritis, etc.

[00297] In certain embodiments, the systemic autoimmune disease is selected from the group consisting of systemic lupus erythematosus, rheumatoid arthritis, systemic vasculitis, scleroderma, pemphigus, dermatomyositis, mixed connective tissue disease, autoimmune hemolytic anemia, thyroid autoimmune disease, and ulcerative colitis.

[00298] In certain embodiments, the autoimmune disease is Graft versus host disease (GvHD).

[00299] In some embodiments, the genetically modified mammalian immune cell or a population thereof of the present disclosure administered to the subject or recipient proliferates *in vivo* and can persist in the subject for an extended period of time.

[00300] In certain embodiment, the subject or recipient is administered with at least 1×10^4 cells, at least 5×10^4 cells, at least 1×10^5 cells, at least 5×10^5 cells, at least 1×10^6 cells, at least 5×10^6 cells, at least 1×10^7 cells, at least 5×10^7 cells, at least 1×10^8 cells, at least 5×10^8 cells, at least 1×10^9 cells, at least 2×10^9 cells, at least 3×10^9 cells, at least 4×10^9 cells, at least 5×10^9 cells, or at least 1×10^{10} cells. In certain embodiment, the subject or recipient is administered at least 1×10^3 cells/kg of bodyweight, at least 5×10^3 cells/kg of bodyweight, at least 1×10^4 cells/kg of bodyweight, at least 5×10^4 cells/kg of bodyweight, at least 1×10^5 cells/kg of bodyweight, at least 5×10^5 cells/kg of bodyweight, at least 1×10^6 cells/kg of bodyweight, at least 5×10^6 cells/kg of bodyweight, at least 1×10^7 cells/kg of bodyweight, at least 5×10^7 cells/kg of bodyweight, at least 1×10^8 cells/kg of bodyweight, at least 2×10^8 cells/kg of bodyweight, at least 3×10^8 cells/kg of bodyweight, at least 4×10^8 cells/kg of bodyweight, at least 5×10^8 cells/kg of bodyweight, or at least 6×10^8 cells/kg of bodyweight.

[00301] A person skilled in the art would understand that dosage of the pharmaceutical compositions provided herein may be determined based on various factors of the subject or recipient, such as size, age, sex, weight, and condition. Dosages can be readily determined by a person skilled in the art from this disclosure and the knowledge in the art.

[00302] The person skilled in the art can readily determine the number of the genetically modified mammalian immune cell provided herein and the amount of optional additives, vehicles, medium and/or carriers in compositions and to be administered in methods of the present disclosure. Typically, additives, if any, are present in an amount of 0.001 to 50% (weight) solution in phosphate buffered saline, and the active ingredient (e.g., the genetically modified mammalian immune cell provided herein) is present in the order of micrograms to milligrams, such as about 0.0001 to about 5 wt%, preferably about 0.0001 to about 1 wt%, still more preferably about 0.0001 to about 0.05 wt%

or about 0.001 to about 20 wt%, preferably about 0.01 to about 10 wt%, and still more preferably about 0.05 to about 5 wt %.

[00303] In certain embodiments, the composition is formulated in a solution comprising 50:50 Plasma-Lyte A to CS10.

[00304] In certain embodiments, the main components is formulated in the cell cryopreservation solution include human serum albumin (HSA), CS10 and Plasma-Lyte A. The cell cryopreservation solution provided herein contains 0.5-5% by weight of HSA, 50-75% by volume of CS10 cryopreservation solution, and 25-50% by volume of an Plasma-Lyte A. Preferably, the cell cryopreservation solution provided herein contains 2.5% by weight of HSA, 50% by volume of CS10 cryopreservation solution, and 40% by volume of Plasma-Lyte A.

[00305] It would be preferred to determine the toxicity of a certain dosage, such as by determining the lethal dose (LD) and LD50 in a suitable animal model (e.g., a mouse). It would also be preferred to determine the timing of administering the composition(s), which elicit a suitable response. Such determinations do not require undue experimentation from the knowledge of the person skilled in the art and the present disclosure.

[00306] Administration of the pharmaceutical compositions provided herein can be performed using various conventional techniques including, without limitation, infusion, transfusion, or parenteral administration. In some embodiments, the parenteral administration includes infusing or injecting intravascularly, intratumorally, intravenously, intradermally, intramuscularly, intraarterially, transtracheally, intrathecally, intraperitoneally, subcutaneously, subcuticularly, intraarticularly, subcapsularly, subarachnoidly and intrasternally.

[00307] In certain embodiments, the pharmaceutical composition provided herein is administered locally to a diseased site (e.g., tumor site). In certain embodiments, the pharmaceutical composition provided herein is administered to a subject by injection, by means of a catheter, by means of a suppository, or by means of an implant (e.g., of a porous, non-porous, or gelatinous material, e.g., a membrane, such as a sialastic membrane). In certain embodiments, the pharmaceutical composition provided herein is delivered in a controlled release system.

[00308] The method treatment provided herein results in an increased survival time of the recipient (e.g., human subject) as compared to the expected survival time of the recipient (e.g., human subject) if the human subject was not treated with the pharmaceutical compositions provided herein. In another embodiment, the increase in the survival time of the human subject is at least 30 days. The increase in the survival time of the human subject can be at least 3 months, at least 6 months

or at least 1 year. In certain embodiments, the recipient (e.g., human subject) to be treated with the methods provided herein is a child (e.g., 0-18 years of age) or an adult (e.g., 18+ years of age).

[00309] In certain embodiments, the treatment methods of the present disclosure relate to using the genetically modified mammalian immune cell or a population thereof that express a CAR. The CAR can be specifically directed towards an antigen target, which is presented on unwanted cells (e.g., cancer cell or auto-reactive lymphocytes) in a host. In certain embodiments, the genetically modified mammalian immune cell has enhanced cytotoxic response against its target. In certain embodiments, the genetically modified mammalian immune cell or a population thereof induces an enhanced cytotoxic response against its target as compared to a reference cell (e.g., native T cell).

[00310] In certain embodiments, the genetically modified mammalian immune cell exhibits an enhanced cytotoxic response by at least 1.2-fold, 1.4-fold, 1.6-fold, 2-fold, 2.5-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 12-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 21-fold, 22-fold, 23-fold, 24-fold, 25-fold, 26-fold or more compared to a reference cell (e.g., native T cell).

[00311] In some embodiments, the genetically modified mammalian immune cell or a population thereof can kill at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 60%, at least 80%, at least 100%, at least 120%, at least 200%, at least 500%, at least 1000% or at least 2000% more target cells than a reference cell (e.g., native T cell). In some embodiments, the genetically modified mammalian immune cell or a population thereof is administered to an allogeneic host, wherein the genetically modified mammalian immune cell or a population thereof has reduced or no (or negligible or minimum) rejection by the host.

[Embodiment 1] A genetically modified mammalian immune cell or a population thereof, comprising a surface molecule and a protection element,

wherein the surface molecule is expressed on the surface of the immune cell, and is capable of binding to one or more target NK receptor ligand (tNKRL) expressed on a target cell thereby activating the immune cell to exert cytotoxic effect on the target cell,

wherein the immune cell, when activated, is capable of being induced to express one or more induced NK receptor ligand (iNKRL) that is capable of binding to the surface molecule, and

wherein the protection element substantially suppresses expression of the one or more induced NK receptor ligand on the immune cell surface following activation of the immune cell, thereby preventing the activated immune cell from becoming a target cell.

[Embodiment 2] The genetically modified mammalian immune cell of Embodiment 1, wherein at least one of the target NK receptor ligands is identical to at least one of the induced NK receptor ligands, or wherein the surface molecule cross-reacts with at least one of the target NK receptor ligands and at least one of the induced NK receptor ligands.

[Embodiment 3] The genetically modified mammalian immune cell of Embodiment 1, wherein the one or more target NK receptor ligand is selected from the group consisting of: NKG2D ligands, NCR ligands, KIR ligands, CD226 ligands, TIGIT ligands, CD96 ligands, or any combination thereof.

[Embodiment 4] The genetically modified mammalian immune cell of Embodiment 1, wherein the one or more target NK receptor ligand comprises one or more NKG2D ligand.

[Embodiment 5] The genetically modified mammalian immune cell of Embodiment 4, wherein the NKG2D ligand is selected from the group consisting of: MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6.

[Embodiment 6] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the expression of the one or more induced NK receptor ligand on the immune cell surface is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%) relative to expression of the one or more induced NK receptor ligand on a reference immune cell surface without the protection element.

[Embodiment 7] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the expression of the one or more induced NK receptor ligand on the immune cell surface is reduced such that the binding the one or more induced NK receptor ligand to the surface molecule is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%).

[Embodiment 8] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the expression of the one or more induced NK receptor ligand is detectable on no more than 50% (e.g., no more than 40%, no more than 30%, no more than 20%, or no more than 10%) of the population of the immune cells following binding of the surface molecule to the one or more target NK receptor ligand.

[Embodiment 9] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the one or more induced NK receptor ligand comprises MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6 and the expression level of the MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6 on the immune cell surface is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%).

[Embodiment 10] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the one or more induced NK receptor ligand comprises MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP5 and ULBP6 and the expression level of each of the MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP5 and ULBP6 on the immune cell surface is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%).

[Embodiment 11] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the immune cell has substantially reduced activation-induced cell death of the immune cell, optionally the reduced activation-induced cell death of the immune cell can be determined by reduced apoptosis detected by Annexin V/7-AAD.

[Embodiment 12] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the immune cell is capable of proliferate upon activation (e.g., having an increased proliferation rate as compared to a modified mammalian immune cell without the protection element).

[Embodiment 13] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the protection element is capable of silencing expression of the one or more induced NK receptor ligand or is capable of inducing intracellular retention and/or degradation of the one or more induced NK receptor ligand.

[Embodiment 14] The genetically modified mammalian immune cell of Embodiment 13, the protection element comprises a small RNA (e.g., an siRNA or miRNA or sh RNA) that targets mRNAs of the one or more induced NK receptor ligand.

[Embodiment 15] The genetically modified mammalian immune cell of Embodiment 13, the protection element comprises one or more protein degradation element that is capable of inducing intracellular retention and/or degradation of the one or more induced NK receptor ligand.

[Embodiment 16] The genetically modified mammalian immune cell of Embodiment 15, wherein at least one of (or all of) the protein degradation elements comprise an endoplasmic reticulum (ER)-retention domain and an iNKRL binding domain capable of binding to the one or more induced NK receptor ligand.

[Embodiment 17] The genetically modified mammalian immune cell of Embodiment 16, wherein the protein degradation element further comprises an ER-retention transmembrane domain, wherein the ER-retention transmembrane domain has one end operably linked to the iNKRL binding domain and the other end operably linked to the ER retention domain.

[Embodiment 18] The genetically modified mammalian immune cell of any one of Embodiments 16-17, wherein the ER-retention domain and/or the ER-retention transmembrane domain is derived from an ER-retention virus protein.

[Embodiment 19] The genetically modified mammalian immune cell of Embodiment 18, wherein the ER-retention virus protein is selected from the group consisting of: HCMV protein US2, HCMV protein US11, HCMV protein US18, HCMV protein US20, HCMV protein US3, HCMV protein US10, HCMV protein US6, HSV ICP47, CPXV12, BHV UL49.5, EBV BNFL2a, HCMV protein UL16, HCMV protein UL141, HCMV protein UL142, HIV Nef, HIV Vpu, HHV-7 U21, HHV-8 KK3, HHV-8 KK5, MHV-68 MK3, HTLV-1 p12, Cowpox Virusprotein CPXV203 and Ad E3/19K.

[Embodiment 20] The genetically modified mammalian immune cell of any one of Embodiments 18-19, wherein the ER-retention domain and the ER-retention transmembrane domain are both derived from the same ER-retention virus protein.

[Embodiment 21] The genetically modified mammalian immune cell of any one of Embodiments 16-20, wherein the iNKRL binding domain comprises an antibody domain (e.g., scFv) capable of binding to the one or more induced NK receptor ligand.

[Embodiment 22] The genetically modified mammalian immune cell of any one of Embodiments 16-20, wherein the iNKRL binding domain is derived from a virus protein capable of binding to the one or more induced NK receptor ligand.

[Embodiment 23] The genetically modified mammalian immune cell of any one of Embodiments 21-22, wherein the one or more induced NK receptor ligand comprises one or more NKG2D ligands.

[Embodiment 24] The genetically modified mammalian immune cell of Embodiment 23, wherein the one or more NKG2D ligands are selected from the group consisting of: MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6.

[Embodiment 25] The genetically modified mammalian immune cell of any one of Embodiments 18-24, wherein the virus protein capable of binding to the one or more NK receptor ligand is derived from a virus selected from the group consisting of: adenovirus (Ad) (e.g., human Ad), cytomegalovirus (CMV) (human CMV, i.e., HCMV) and Kaposi's sarcoma-associated herpesvirus (KSHV).

[Embodiment 26] The genetically modified mammalian immune cell of any one of Embodiments 18-25, wherein the virus protein capable of binding to the one or more NK receptor ligand is derived from a virus protein selected from the group consisting of: Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), HCMV protein UL16, HCMV protein UL142, HCMV protein US18, HCMV protein US20, and KSHV K5.

[Embodiment 27] The genetically modified mammalian immune cell of any one of Embodiments 22-26, wherein the ER-retention domain and the binding domain are both derived from the same virus protein.

[Embodiment 28] The genetically modified mammalian immune cell of any one of Embodiments 22-26, wherein the ER-retention domain, the ER-retention transmembrane domain and the binding domain are all derived from different virus protein.

[Embodiment 29] The genetically modified mammalian immune cell of Embodiment 27, wherein the same virus protein is selected from the group consisting of Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), HCMV protein UL16, HCMV protein UL142, HCMV protein US18, HCMV protein US20, KSHV K5.

[Embodiment 30] The genetically modified mammalian immune cell of any one of Embodiments 18-27 and 29, wherein the protein degradation element comprises a virus protein selected from the group consisting of: Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), HCMV protein UL16, HCMV protein UL142, HCMV protein US18, HCMV protein US20, KSHV K5 or an analog thereof, or a derivative thereof.

[Embodiment 31] The genetically modified mammalian immune cell of Embodiment 14, wherein the protection element comprises a small RNA, such as HCMV encoded microRNAs (miR-20a, miR-UL112) are able to modulate NKG2D-ligand expression.

[Embodiment 32] The genetically modified mammalian immune cell of any one of Embodiments 18-30, wherein the one or more protein degradation elements comprise a combination of virus proteins, wherein the combination of virus proteins comprises a HCMV protein UL16 and another virus

protein selected from the group consisting of: Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), HCMV protein UL142, HCMV protein US18, HCMV protein US20, and KSHV K5.

[Embodiment 33] The genetically modified mammalian immune cell of any one of Embodiments 15-32, wherein the one or more protein degradation elements further comprise a protein degradation pathway (PDP) binding domain capable of binding to a member in a protein degradation pathway.

[Embodiment 34] The genetically modified mammalian immune cell of Embodiment 33, wherein the PDP binding domain is operably linked to the ER-retention domain.

[Embodiment 35] The genetically modified mammalian immune cell of any one of Embodiments 33-34, wherein the protein degradation pathway is ubiquitylation-proteosome pathway, endosome-lysosome pathway, or autophagic degradation pathway.

[Embodiment 36] The genetically modified mammalian immune cell of Embodiment 35, wherein the member in the ubiquitylation-proteosome pathway comprises E1 ubiquitin activating enzyme, E2 ubiquitin conjugating enzyme, or E3 ubiquitin ligase.

[Embodiment 37] The genetically modified mammalian immune cell of Embodiment 35, wherein the member in the endosome-lysosome pathway comprises AP-1, AP-2, AP-3, endosome, lysosome, HOPS, ESCRT, GASP, BLOC-1, ESCRT, Retromer, ESCRT, sortingnexin, Dapper2, SNX4, Pincher, Rap1-PDZ-GEF1, clathrin, or C3G/CrkL/Shp2/Gab2.

[Embodiment 38] The genetically modified mammalian immune cell of Embodiment 35, wherein the member in the autophagic degradation pathway comprises chaperone-mediated autophagy (CMA) USP10, G3BP1, ULK1, ATG16L1, TRIM16, FBXO27VDAC, RHOT1, MFN1/2, BNIP3L, FUNDC1, BNIP3, AMBRA1, BCL2LI3, FKBP8, CHDH, DISC1, PHB2, Cardiolipin, SEC62, RTN3, PEX5, PEX14, ABCD3, or NUFIP1.

[Embodiment 39] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the surface molecule comprises a) a tNKRL-binding extracellular domain capable of binding to the one or more target NK receptor ligand, and b) a surface molecule (SM) intracellular domain capable of activating the immune cell following binding of the tNKRL-binding extracellular domain to the one or more target NK receptor ligand, and wherein the tNKRL-binding extracellular domain is associated with the SM intracellular domain to allow transduction of binding signal from the tNKRL-binding extracellular domain to the SM intracellular domain.

[Embodiment 40] The genetically modified mammalian immune cell of Embodiment 39, wherein the tNKRL-binding extracellular domain comprises an antibody domain that is capable of binding to the one or more target NK receptor ligand.

[Embodiment 41] The genetically modified mammalian immune cell of Embodiment 39, wherein the tNKRL-binding extracellular domain comprises an NK receptor extracellular domain that is capable of binding to the one or more target NK receptor ligand.

[Embodiment 42] The genetically modified mammalian immune cell of Embodiment 39, wherein the tNKRL-binding extracellular domain comprises an NKG2D extracellular domain.

[Embodiment 43] The genetically modified mammalian immune cell of Embodiment 41, wherein the receptor further comprises a SM transmembrane domain, wherein the SM transmembrane domain has one end operably linked to the tNKRL-binding extracellular domain and the other end operably linked to the SM intracellular domain.

[Embodiment 44] The genetically modified mammalian immune cell of Embodiment 43, wherein the SM transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of CD3, CD4, CD8, CD28, B7, ICOS, CD226, 41-BB, OX40, CD27, GITR, HVEM, CD40, BAFFR, BAFF, NKG2D, NKG2C, NKG2A, NKp44, NKp46, NKp30.

[Embodiment 45] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the surface molecule comprises a tNKRL-binding chimeric antigen receptor (CAR).

[Embodiment 46] The genetically modified mammalian immune cell of Embodiment 45, wherein the receptor comprises an CNK receptor protein complex comprising a first polypeptide comprising the tNKRL-binding extracellular domain operably linked to a first CNK transmembrane domain, and a second polypeptide comprising the CNK intracellular domain operably linked to a second CNK transmembrane domain, wherein the first CNK transmembrane domain and the second CNK transmembrane domain associate with each other to form the protein complex.

[Embodiment 47] The genetically modified mammalian immune cell of Embodiment 46, wherein the first CNK transmembrane domain comprises a NK receptor transmembrane domain, optionally an NKG2D transmembrane domain.

[Embodiment 48] The genetically modified mammalian immune cell of Embodiment 46, wherein the first transmembrane domain is capable of forming a first dimer.

[Embodiment 49] The genetically modified mammalian immune cell of Embodiment 46, wherein the first polypeptide comprises a NKG2D protein.

[Embodiment 50] The genetically modified mammalian immune cell of Embodiment 49, wherein the NKG2D protein comprises the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2.

[Embodiment 51] The genetically modified mammalian immune cell of any one of Embodiments 46-50, wherein the second transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of DNAX-Activating Protein of 10 kDa (DAP10) and DNAX-Activating Protein of 12 kDa (DAP12).

[Embodiment 52] The genetically modified mammalian immune cell of any one of Embodiments 46-51, wherein the CNK intracellular domain comprises a cytoplasmic domain of a protein selected from the group consisting of: 4-1BB (CD137), CD2, CD3, CD35, CD25, CD27, CD28, CD30, CD40, CD79A, CD79B, CARD11, DAP10, DAP12, Fc receptor, Fyn, LIGHT, LT β R, HVEM, ICOS, Lck, LAG3, LAT, LRP, NOTCH1, NOTCH2, NOTCH3, NOTCH4, OX40 (CD134), ROR2, Ryk, SLAMF1, Slp76, pTa, TCR α , vTCRP, TRIM, Zap70, PTCH2, IL7 receptor, IL15 receptor and GITR.

[Embodiment 53] The genetically modified mammalian immune cell of any one of Embodiments 46-51, wherein the CNK intracellular domain comprises a cytoplasmic domain of CD3 (e.g., CD3 zeta).

[Embodiment 54] The genetically modified mammalian immune cell of any one of Embodiments 46-53, wherein the CNK intracellular domain comprises the amino acid sequence of SEQ ID NO: 5.

[Embodiment 55] The genetically modified mammalian immune cell of any one of Embodiments 46-54, wherein the second polypeptide comprises a chimeric adaptor protein comprising:

- a) the transmembrane domain of DAP10 operably linked to the intracellular domain of CD3 (e.g., CD3 zeta);
- b) the transmembrane domain of DAP12 operably linked to the intracellular domain of CD3 (e.g., CD3 zeta); or
- c) the transmembrane domain of DAP10 operably linked to the intracellular domain of DAP12.

[Embodiment 56] The genetically modified mammalian immune cell of any one of Embodiments 46-54, wherein the second polypeptide comprises a full-length DAP10 operably linked to the intracellular domain of CD3 zeta.

[Embodiment 57] The genetically modified mammalian immune cell of Embodiment 55, wherein the chimeric adaptor protein comprises the amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 16.

[Embodiment 58] The genetically modified mammalian immune cell of any one of Embodiments 46-56, wherein the second CNK transmembrane domain is capable of forming a second dimer, and wherein the second dimer associates with the first CNK transmembrane domain to form the complex.

[Embodiment 59] The genetically modified mammalian immune cell of any one of Embodiments 46-56, wherein the CNK receptor comprises a hexameric protein complex comprising a homodimer of the NKG2D protein and two homodimers of the chimeric adaptor protein.

[Embodiment 60] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the immune cell is further modified to be deficient in a) MHC class I protein, or b) MHC class II protein or MHC class II transactivator, or c) both a) and b).

[Embodiment 61] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the immune cell is T cell and the immune cell is further modified to be deficient in endogenous TCR.

[Embodiment 62] The genetically modified mammalian immune cell of any one of the preceding Embodiments, further expressing a target binding (TB) receptor capable of binding to a target marker.

[Embodiment 63] The genetically modified mammalian immune cell of Embodiment 62, wherein the target marker is expressed on the target cell.

[Embodiment 64] The genetically modified mammalian immune cell of Embodiment 63, wherein the TB receptor comprises a target-marker binding extracellular domain capable of binding to the target marker and a TB intracellular domain capable of activating the immune cell upon binding of the target-marker binding extracellular domain to the target marker.

[Embodiment 65] The genetically modified mammalian immune cell of Embodiment 64, wherein the TB receptor further comprises a TB transmembrane domain.

[Embodiment 66] The genetically modified mammalian immune cell of Embodiment 65, wherein the TB transmembrane domain has one end operably linked to the target-marker binding extracellular domain and the other end operably linked to the TB intracellular domain.

[Embodiment 67] The genetically modified mammalian immune cell of Embodiment 66, wherein the target-marker binding extracellular domain comprises the extracellular domain of a natural cytotoxicity receptor (e.g., NKp30 (NCR3), NKp44 (NCR2), and NKp46 (NCR1)).

[Embodiment 68] The genetically modified mammalian immune cell of Embodiment 67, wherein the TB transmembrane domain comprises the transmembrane domain of a protein selected from the group consisting of CD3, CD4, CD8, CD28, B7, ICOS, CD226, 41-BB, OX40, CD27, GITR, HVEM, CD40, BAFFR, BAFF, NKG2D, NKG2C, NKG2A, NKp44, NKp46, NKp30.

[Embodiment 69] The genetically modified mammalian immune cell of Embodiment 67 or 68, wherein the TB intracellular domain comprises a cytoplasmic domain of a protein selected from the group consisting of: 4-1BB (CD137), CD2, CD3, CD35, CD25, CD27, CD28, CD30, CD40, CD79A, CD79B, CARD11, DAP10, DAP12, Fc receptor, Fyn, LIGHT, LT β R, HVEM, ICOS, Lck, LAG3, LAT, LRP, NOTCH1, NOTCH2, NOTCH3, NOTCH4, OX40 (CD134), ROR2, Ryk, SLAMF1, Slp76, pTa, TCR α , vTCRP, TRIM, Zap70, PTCH2, IL7 receptor, IL15 receptor and GITR.

[Embodiment 70] The genetically modified mammalian immune cell of Embodiment 69, wherein the TB intracellular domain comprises a cytoplasmic domain of CD28 operably linked to CD3 zeta chain.

[Embodiment 71] The genetically modified mammalian immune cell of any one of Embodiments 62-70, wherein the target marker is a tumor antigen, an auto-reactive cell antigen or a virus antigen, bacteria antigen, a microbe-infected cell, or damaged and/or aged cells.

[Embodiment 72] The genetically modified mammalian immune cell of any one of Embodiments 63-70, wherein the target cell is a tumor cell, an auto-reactive cell, a senescent cell or a virus-infected cell.

[Embodiment 73] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the immune cell is a T cell, a Natural Killer (NK) cell, a NKT cell, a B cell, a hematopoietic cell (e.g., bone marrow cell), a thymocyte, a dendritic cell (e.g., a mature dendritic cell), a macrophage cell, a tumor infiltrating lymphocyte, a monocyte, a granulocyte.

[Embodiment 74] The genetically modified mammalian immune cell of any one of the preceding Embodiments, wherein the immune cell is a T cell, such as CD4⁺ T cell, CD8⁺ T cell, cytotoxic T cell, terminal effector T cell, memory T cell, na ν e T cell, natural killer T cell, gamma-delta T cell, cytokine-induced killer (CIK) T cell, or tumor infiltrating lymphocyte.

[Embodiment 75] A pharmaceutical composition comprising the genetically modified mammalian immune cell or the population thereof.

[Embodiment 76] A method for treating a disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of Embodiment 75.

[Embodiment 77] The method of Embodiment 76, wherein the disease is cancer, a disease associated with auto-reactive lymphocytes (e.g., an autoimmune disease), an immune deficient disease, or an aging associated disease.

[Embodiment 78] The method of Embodiment 77, wherein the autoimmune disease is organ-specific autoimmune disease or systemic autoimmune disease.

[Embodiment 79] The method of Embodiment 78, wherein the organ-specific autoimmune disease is selected from the group consisting of chronic lymphocytic thyroiditis, hyperthyroidism, insulin-dependent diabetes mellitus, myasthenia gravis, ulcerative colitis, pernicious anemia with chronic atrophic gastritis, pulmonary hemorrhage nephritic syndrome, pemphigus vulgaris, pemphigoid, primary biliary cirrhosis, multiple sclerosis, acute idiopathic polyneuritis, etc.

[Embodiment 80] The method of Embodiment 78, wherein the systemic autoimmune disease is selected from the group consisting of systemic lupus erythematosus, rheumatoid arthritis, systemic vasculitis, scleroderma, pemphigus, dermatomyositis, mixed connective tissue disease, autoimmune hemolytic anemia, thyroid autoimmune disease, and ulcerative colitis.

[Embodiment 81] The method of Embodiment 77, wherein the autoimmune disease is Graft versus host disease (GvHD).

[Embodiment 82] An expression construct encoding a surface molecule and a protection element, wherein the construct can be expressed in an immune cell, wherein the surface molecule is as defined in any one of Embodiments 43-49, and the protection element is as defined in any one of the Embodiments 17-42.

[Embodiment 83] A method of producing the genetically modified mammalian immune cell of any one of the Embodiments 1-74, comprising introducing the expression construct of Embodiment 82 to a starting immune cell under conditions to allow expression of the surface molecule on the surface of the immune cell and to allow expression of the protection element in the immune cell.

EXAMPLES

[00312] While the disclosure has been particularly shown and described with reference to specific embodiments (some of which are preferred embodiments), it should be understood by those having skill in the art that various changes in form and detail may be made therein without departing from the spirit and scope of the present disclosure as disclosed herein.

[00313] **Example 1. Activated T cells significantly increased the expression of NKG2D ligands.**

[00314] T cells were activated with Dynabeads® Human T-Activator CD3/CD28 (Thermo Scientific) (Microbeads : T cell=1: 1) for 2 days and then submitted to flow cytometry to examine the expression of NKG2D ligands (MICA, MICB, ULBP1-6). The result indicates MICA, MICB and ULBP2, 5, 6, ULBP3 have been upregulated in activated T cells, except ULBP4. (FIG. 1A and FIG. 1B).

[00315] **Example 2. Production of lentivirus encoding chimeric natural killer receptor and CNK-T cells**

[00316] To prepare the virus solution, CNK plasmid (NKG2D/NCR2) (constructed by GENEWIZ) expressed the specific chimeric natural killer receptor (illustrated in FIG. 2A), along with the package plasmids gal-pol, Rev and VSVG (OBIO), were mixed with transfection reagent PEI pro (Polyplus) in a ratio of CNK:gal-pol:rev:vsvg=12:5:4:4 and then transfect into 293T cells in a 1:1 mixture. After four hours of transfection, OptiPRO SFM medium (Thermofisher) was added to replace the complete medium. The supernatant was collected after 48 hours of downstream purification.

[00317] The clarified filtrate medium containing virus was filtered by Diamond Layer 400, followed by ultrafiltration concentration. After ultrafiltration concentration, add a 25% concentration ratio of sucrose solution to 10% of the harvested solution volume to obtain the final product (containing 2.5% concentration ratio of sucrose. After that, lentivirus was stored at $-70 \pm 10^{\circ}\text{C}$.

[00318] Human CD8⁺ and CD4⁺ were isolated from PBMC of normal donors using CD3⁺ T Cell Isolation Kit (Miltenyi Biotec), activated with anti-CD3/CD28 beads (Life Technologies) according to the manufacturer's instructions, and transduced with a lentiviral vector encoding CNK, CNK+PE (UL16) and CNK+PE/NCR2 ED-CD28 TMD-CD28 ICD-CD3Z ICD respectively, with 0.8 µg/mL polybrene (Millipore, Bedford, Mass.) on day 3 after activation by centrifugation at 2,100 rpm for 45 min at 32°C. T cells were expanded in RPMI, 10% human serum, 2 mM L-glutamine and 1% penicillin-streptomycin (CTL medium), supplemented with recombinant human (rh) IL-2 to a final concentration of 50 U/mL every 48 hours. After 12 days' expansion, an aliquot of each transduced T cell line was stained with CD8-Pacific Blue, NKG2D-PE-Cy7, NKp44-PE, 7-AAD (Biolegend) and then submitted to flow cytometry analysis. The results show introduction of CNK elements could significantly improve expression of NKG2D in CD4 as well as

CD8 population. Moreover, T cells transduced with the lentiviral vector encoding CNK+PE/NCR2-CD28-CD3Z express both NKG2D and NKP44. Interestingly, the FSC/SSC flow plot indicates introduction of PE (UL16) could improve the survival percentage of cells in the culture (Fig. 2B).

[00319] Example 3. The protection element (PE) could inhibit NKG2D ligand expression on the CNK-T cells.

[00320] T cells were activated with Dynabeads® Human T-Activator CD3/CD28 (Thermo Scientific) (M:T=3:1) for 2 days, then transduced with lentiviral vector encoding CNK and protective elements (UL16), and expanded as previous description. T cells were submitted to flow cytometry after staining by NKG2D ligand antibody (Biolegend) or NKG2D-Fc recombinant protein (SGE Biotech) to examine the expression of NKG2D ligands (MICA, MICB, ULBP1-6) at Day 2, Day 4 and Day 6 of culture. The nontransduced T cells were used as control. The result indicates the protective elements could significantly suppress NKG2D ligands expressed on the T cells surface during activation (FIG. 3).

[00321] Example 4. The protection element (PE) could increase CNK-T cell viability and proliferation.

[00322] T cells were activated with Dynabeads® Human T-Activator CD3/CD28 (Thermo Scientific) for 2 days, then transduced with lentiviral vector encoding CNK elements (CNK-T), CNK elements with PE element (UL16) (CNK+PE), the nontransduced T cells were used as control. The cells were submitted to automated cell counter (Countstar) to examine the viability and number at Day2, 4, 6, 8, 10 and 12. The result indicates the protective elements could improve cell viability during early stage of cell expansion and promote cell proliferation. Including UL16 could significantly improve CNK-T survival and proliferation (FIG. 4).

[00323] Example 5. Preparation and characterization of NCR2 CNK-UT cells

[00324] The NCR2 CNK-UT cells derived from healthy donor T cells are generated using lentivirus and CRISPR/Cas9 genome-editing technology. Briefly, donor CD3+T cells were activated with TransAct(Miltenyi Biotec) and cultured at 37℃ and 5% CO₂. Next day, the lentivirus vector encoding CNK+PE and NCR2 ED-CD28 TMD-CD28 ICD-CD3z ICD was thawed from a -80℃ freezer at room temperature and added to the cell mixture of MOI=1, centrifuged at 1000×g for 60 minutes at 32℃, and then cultured overnight at 37℃ and 5% CO₂. The NCR2 CNK-UT cells derived from healthy donor T cells are generated using lentivirus and CRISPR/Cas9 genome-editing technology. The CNK-UT cells derived from healthy donor T cells are generated using lentivirus and

CRISPR/Cas9 genome-editing technology. Cells were collected and washed twice with D-PBS (Thermofisher) before being resuspended in opti-MEM(Thermofisher) at a density of $5 \sim 10 \times 10^6$ /ml. A ribonucleoprotein (RNP) complex was prepared by incubating cas9 protein and sgRNA targeting TRAC, B2M, and CIITA as described previous (Yuki Kagoya et al., 2020) at room temperature for 15 minutes before electroporated into the cells using X-Porator H1 (Etta Biotech). Following electroporation, the cells were immediately cultured at 37 °C and 5% CO₂ for 7 days. Residual TCR+ cells were depleted using CliniMACS. The cells were submitted to flow cytometry for analysis. The result indicates expressed NKG2D on both CD8+ and CD4+ T cells, the expression level is significantly higher than the control T cells, which only express NKG2D on CD8+ T cells. 91.7% NCR2 CNK-UT cells also express NCR2 (Nkp44). HLA class II, TCR and b2M knockout efficiency was quantified by flow cytometry as well. Before selection by CliniMACS, 91.5% of cells are TCR $\alpha\beta$ and CD3 negative. After CD3 negative selection, up to 99.3% cells are TCR $\alpha\beta$ and CD3 negative. In addition, 91.9% and 88.4% of cells were HLA-DR and HLA-abc negative respectively (FIG. 5).

[00325] Example 6. UT efficiently eliminated activated T cells and IL2 does not protect activated T cells from attack by NCR2 CNK-UT.

[00326] T cells were activated with Dynabeads® Human T-Activator CD3/CD28 (Thermo Scientific) for 2 days and then co-cultured with NCR2 CNK-UT at the effect-to-target ratio E:T=3:1, and the nontransduced T cells as a control. After 72h incubation, the cells were collected and submitted for flow cytometry. TCR knockout via CRISPR-Cas9 gene editing leads to defect of CD3 expression on NCR2 CNK-UT cells. Therefore, it is easy to distinguish NCR2 CNK-UT cells (TCR-, CD3-), control and activated T cells (TCR+, CD3+). Flow cytometry showed that NCR2 CNK-UT cells could efficiently eliminate activated T cells, while control T cells could not deplete activated T cells. (Fig. 6).

[00327] Example 7. UT cells efficiently eliminated activated T cells, but not the rest T cells.

[00328] To examine whether NCR2 CNK-UT exhibit cytotoxicity against the resting T cells, the activated T cells were co-incubated with NCR2 CNK-UT cells at the E:T ratio of 3:1 for 48h. Then the cells were collected and submitted to flow cytometry. The result indicates that in the group of NCR2 CNK-UT co-culture with activated T cells, CD3(+)TCR(+) cells were complete absent after 48h coculture. Interestingly, in the group of NCR2 CNK-UT co-culture with inactivated T cells, CD3(+)TCR(+) cells were still present after 48h. It suggests NCR2 CNK-UT could only efficiently eliminate activated T cells but not the resting T cells (Fig.7).

[00329] Example 8. Jurkat cells express NKG2D ligands and NCR ligands

[00330] T-ALL cell line Jurkat cells were submitted to flow cytometry to examine the expression of NKG2D ligands (MICA, MICB, ULBP1-6) using the antibodies (Biolegend), NKG2D-Fc and different NCR-Fc recombinant protein (SGE Biotech). The IgG isotypes (Biolegend) were used as control. The results suggest Jurkat cells highly express ULBP-1 and ULBP2,5,6, mildly express MICA and MICB, except ULBP-3 and ULBP-4. Both NKG2D-Fc and NKp44L could efficiently bind to Jurkat cells. (Fig.8).

[00331] Example 9. NCR2 CNK-UT cells efficiently kill Jurkat T cells

[00332] To examine whether NCR2 CNK-UT exhibit cytotoxicity against the Jurkat T cells, the NCR2 CNK-UT cells were co-incubated with Jurkat T cells at the E:T ratio of 1:2, 1:1 and 2:1 for 48h, the control T cells were used as control. The NCR2 CNK-UT cells have been knockout TCR, so CD3 expression was absent compared to Jurkat T cells (CD3+, CD8-). After 48h coculture, the cocultured cells were collected and submitted to flow cytometry. The result indicates that in the group of NCR2 CNK-UT co-culture, CD3(+)CD8(-) cells were almost absent after 48h coculture. (Fig.9).

[00333] Example 10. The mechanism of CNK-UT to treat T-cell mediated autoimmune diseases

[00334] Fig10A. Allogeneic or auto-reactive T cells recognize specific antigen presented by MHC molecules (eg. HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR) on tissue cells; During the T cells response, the activated allogeneic or autoreactive T cells upregulate NKG2D ligands (eg. MICA/B, ULBP1-6); Then the allogeneic or autoreactive T cells will attack and destroy the tissue cells. Fig.10B. CNK-UT cells have been introduced with CNK elements (NKG2D) and knockout TCR which completely prevent TCR-mediated GVHD. During the T cells response, the activated allogeneic or autoreactive T cells upregulate NKG2D ligands which can be recognized by CNK elements in CNK-UT. CNK-UT could recognize and directly kill activated allogeneic or autoreactive T cells dependent on NKG2DL and protect the tissue cells from T cells-mediated autoimmunity.

[00335] Example 11. Scheme of GVHD experimental protocol

[00336] Twenty mice were intraperitoneally injected with 30mg/kg of busulfan which is defined as day 0; Each of the sixteen mice was injected with 200 μ L of PBMCs, 5E6 cells each time on the first and second days; On day 4, each of the eight mice was i.v. infused with 1E7 NCR2 CNK-

UT cells, followed by GVHD symptom observation 3 times a week and blood collection for flow analysis every week (Fig.11).

[00337] Example 12. NCR2 CNK-UT efficiently eliminates allogeneic T leukocytes in GVHD mice.

[00338] Knockout TCR in NCR2 CNK-UT has resulted in CD3 deficient in T cells, so the UT cells are CD45(+)CD3(-), and the T cells in allogeneic PBMC were CD45(+)CD3(+), while the peripheral blood cells of mice were CD45(-)CD3(-). The result shows, the percentage of CD45(+)CD3(+) cells in PBL was significantly increased during GVHD development in mice. The allogeneic T cells can expand up to ~50% of total PBL after 3 weeks of PBMC infusion in GVHD mice. Interestingly, in the GVHD mice treated with NCR2 CNK-UT, the CD45(+) CD3(+) cells were completely eradicated by NCR2 CNK-UT cells. NCR2 CNK-UT could reduce the activated allogeneic T cells in PBL which is almost undetectable as the control mice without PBMC infusion after 3 weeks treatment. In conclusion, NCR2 CNK-UT infusion could efficiently inhibit the development of GVHD, suppress allogeneic human T cells expansion and finally eliminate all the allogeneic T cell in GVHD mice (FIGs. 12A-12D).

[00339] Example 13. NCR2 CNK-UT treatment could prevent the development of GVHD in mice.

[00340] We observed a characteristic pattern of physical symptoms of GvHD including weight loss, skin desquamation, hunching and loss of activity, diarrhea in nontreated mice. Mice were weighed and monitored weekly for visible signs of GVHD, with scoring carried out as Table 1. Activity and posture of NCR2 CNK-UT treated mice will remain relatively normal post-transplant. Cumulative GVHD scores will steadily increase through the first 2-3 weeks post-transplant in the non-treated mice. Interestingly, NCR2 CNK-UT-treated animals showed a significantly diminished GvHD score as shown in Fig. 14.

[00341] Table 1. GVHD scoring criteria.

Index	0	1	2
Weight loss	<10%	>10%~25%	>25%
Posture	Regular	When there is no activity, the back is bowed, and when there is activity, it disappears	Severe arched back, affecting the movement

Activity	Regular	Decreased range of motion, but voluntary activity	Activity is when it's exciting
Body hair texture	Regular	Light to moderate shrugging	Severe shrugging
Skin integrity	Regular	Single small lesions	Multiple extensive lesions
If diarrhea or anal swelling is found, 2 points will be added.			

[00342] **Example 14. NCR2 CNK-UT treatment could prevent the weight loss in GVHD mice**

[00343] GVHD mice will also begin to lose weight between 7-10 days post PBMC infusion. The GVHD mice lost up to 20% weight compared to the control mice, in contract, the mice with NCR2 CNK-UT treatment started to gain weight as the normal mice. (Fig.15).

[00344] **Example 15. NCR2 CNK-UT treatment could significantly improve the survival of mice suffering from GVHD**

[00345] Importantly, symptoms further improved over time in non-treated mice and only 25% mice survive after 40 days after PBMC infusion. In contract, NCR2 CNK-UT-treatment stay alive as long as the control T cells. Therefore, the result indicate NCR2 CNK-UT could efficiently protect the mice development of GVHD as well as improve survival of the GVHD mice after PBMC infusion. The median survival time in NKG2D mice without treatment was significantly shorter than that of the NCR2 CNK-UT-treated mice. (Fig. 16).

[00346]

Table 2. Sequences mentioned or used in the present application

SEQ ID NO	Sequence	Annotation
1	MGWIRGRRSRHSWEMSEFHNYNLDLKKSDFSTRWQKQRCPV VKSKCRENASPFFCCFIAVAMGIRFIIMVTIWSAVFLNSLFNQE VQIPLTESYCGPCPKNWICYKNNCYQFFDESKNWYESQASCMS QNASLLKVYSKEDQDLLKLVKSYHWMGLVHIPTNGSWQWED GSILSPNLLTIEMQKGDCALYASSFKGYIENCSTPNTYICMQRT V	Human NKG2D
2	MGWIRGRRSRHSWEMSEFHNYNLDLKKSDFSTRWQKQRCPV VKSKCRENASPMFVVRVLAIALAIRFTLNTLMWLAIKFETFQPV LFNQEVQIPLTESYCGPCPKNWICYKNNCYQFFDESKNWYESQ ASCMSQNASLLKVYSKEDQDLLKLVKSYHWMGLVHIPTNGSW QWEDGSILSPNLLTIEMQKGDCALYASSFKGYIENCSTPNTYIC MQRTV	chimeric human NKG2D with mouse NKG2D transmembrane (hmcNKG2D)
3	MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGL SLPLLAGLVAAADAVASLLIVGAVFLCARPRRSPAQDGKVYINM PGRG	DAP10
4	MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSPGVL AGIVMGDLVLTVLIALAVYFLGRLVPRGRGAAEAATRKQRITE TESPYQELQGQRSDVYSDLNTQRPYYK	DAP12
5	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR	CD3 ζ ICD
6	EPKSCDKTHTCPPCP	IgG1 hinge
7	ESKYGPPCPSCP	IgG4 hinge
8	LVAADAVASLLIVGAVF	DAP10 transmembrane domain
9	GVLGIVMGDLVLTVLIALAV	DAP12 transmembrane domain

10	GVLAGIVMGDLVLTVLIALAVLVAADAVASLLIVGAVF	DAP10- DAP12 transmembrane domain fusion
11	GSGEGRGSLTCDVEENPGP	T2A
12	MGWIRGRRSRHSWEMSEFHNYNLDLKKSDFSTRWQKQRCPV VKSKCRENASP	Human NKG2D intracellular domain
13	GGGGS	Linker
14	IWSAVFLNSLFNQEVQIPLTESYCGPCPNWICYKNNCYQFFDE SKNWYESQASCMSQNASLLKVYSKEDQDLLKLVKSYHWMGL VHIPTNGSWQWEDGSILSPNLLTIEMQKGDALYASSFKGYIE NCSTPNTYICMQRTV	Human NKG2D extracellular domain
15	MIHLGHILFLLLPVAAAQTTPGERSSLPFYPGTSGSCSGCGSL SLPLLAGLVAADAVASLLIVGAVFLCARPRRSPAQDGKVYINM PGRGGGGSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV <u>LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM</u> <u>KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR</u>	DAP10-CD3Z ICD
16	MIHLGHILFLLLPVAAAQTTPGERSSLPFYPGTSGSCSGCGSL SLPLLAGLVAADAVASLLIVGAVFLCARPRRSPAQDGKVYINM PGRGGGGSYFLGRLVPRGRGAAEAATRKQRITETESPYQELQG QRSDVYSDLNTQRPYYK	DAP10- DAP12CD
17	MNNLWKAWVGLWTSMGPLIRLPDGITKAGEDALRPWKSTAK HPWFEIEDNRCYIDNGKLFARGSIVGNMSRFVFDPKADYGGVG ENLYVHADDVEFVPGESLKWNVRNLDVMPIFETLALRLVLQG DVIWLRCPPELRVDYTSSAYMWNMQYGMVRKSYTHVAWTIV FYSINITLLVLFIVYVTDCNLSMMWMRFFVC	HCMV US2
18	MNLVMLILALWAPVAGSMPELSLTLFDEPPPLVETEPLPPLSDV SEYRVEYSEARCVLRSGGRLEALWTLRGNLSVPTPTPRVYYQT LEGYADRVPTPVEDVSESLVAKRYWLRDYRVPQRTKLVLFFYS PCHQCQTYYYVECEPRCLVPWVPLWSSLEDIERLLFEDRRLMAY	HCMV US11

	YALTIKSAQYTLMMVAVIQVFWGLYVKGWLHRHFPWMFSDQ W	
19	MGDTASVSEHHESPTVTIVPLHRSHALVAEQQLFQWLKRFKLL MEVYHGLVWQLACTLTVCLLAWLAFPDVQGGCANGIVPALSS IVPVSTLAMLRGFAEFRPHTTNFAHLTVACLLINTGITVCTGFCG ERRVIGLSFALVMVFFVLCSGLTYLAGNNPTRWKVIGIGYGWS VIVFYLLLYFSPVLWVSKIYSGLYVLVVTAASAVLIYETLDLIY QRGTLKNSVCVSVVLYTIVMSLLNMSVAIFSGHVWVQQYAE KHGGRIDGVSLLSLL	HCMV US18
20	MQAQEANALLSRMEALEWFKKFTVWLRVYAIFIFQLAFSFG GSVFWLGFPQNRNFCVENYSFFLTVLVPIVCMFITYTLGNEHPS NATVLFIFYLLANSLTAAIFQMCSESRVLVGSYVMTLALFISFTGL AFLGGRDRRRWKCISCYVVMMLLSFLTALLSDADWLQKIVVT LCAFSISFFLGILAYDSLMVIFFCPPNQCIRHAVCLYLDMAIFLT LLLMLSGPRWISLSDGAPLDNGTLTAASTTGKS	HCMV US20
21	MKPVLVLAILAVLFLRLADSVPRPLDVVVSEIRSAHFRVEENQC WFHMGMLYFKGRMSGNFTEKHFFVNVGIVSQSYMDRLQVSGE QYHHDERGAYFEWNIGGHPVTHTVDMVDITLSTRWGDPPKYA ACVPQVRMDYSSQTINWYLQRSMRDDNWGLLFRTLLVYLFSL VVLVLLTVGV SARLRFI	HCMV US3
22	MLRRGSLRNPLAICLLWWLGVVAAATEETREPTYFTCGCVIQN HVLKGAVKLYGQFPSPKTLRASAWLHDGENHERHRQPILVEGT ATATEALYILLPTELSSPEGNRPRNYSATLTLASRDCYERFVCPV YDSGTPMGVLMNLTYLWYLGDYGAILKIYFGLFCGACVITRSL LLICGYPPRE	HCMV US10
23	MDLLIRLGFLLMCALPTPGERSSRDPKTLTSLSPRQQACVPRTK SHRPVCYNDTGDCTDADDSWKQLGEDFAHQCLQAAKKRPKT HKSRPNDRNLEGRLTCQVRRLPCDLDIHPSHRLLTLMNNCV CDGAVWNAFRLIERHGFFAVTLYLCCGITLLVVILALLCSITYES TGRGIRRCGS	HCMV US6
24	MSWALEMADTFLDTMRVGPRTYADV RDEINKRGREDREAAR TAVHDPERPLLRSPGLLPEIAPNASLGVAHRRTGGTVTDSRNP VTR	ICP47

25	MFIMRELVYRVVIVILSLSLLSSFLVICFIEHRCFKEETRYKICPK NTISINNICYFSEDTTNWTASFEYCKNMNGRLACIINDTFPKIC RYKGTFDYWVGLYRTNSQSPWLNCNKPYNIIPIRGVEDYAY LNNNGISTARIYADRRWICEVPMVDY	CPXV12
26	MPRSPLIVAVVAAALFAIVRGRDPLLDAMRREGAMDFWSAGC YARGVPLSEPPQALVVFYVALTAVMVAVALYAYGLCFRLMGA SGPNKKESRGRG	BHV UL49.5
27	MVHVLERALLEQQSSACGLPGSSTETRPSHPCPEDPDVSRLRL LAVLCVLFGLLCLLI	EBV BNFL2a
28	MERRRGTVPLGWVFFVLCLSASSSCAVDLGSKSSNSTCRLNVT ELASIHGETWTLHGMCISICYENVTEDEIIGVAFTWQHNE VDLWLYQNDTVIRNFSDITTNILQDGLKMRTVPVTKLYTSRMV TNLTVGRYDCLRCENGTTKIERLYVRLGSLYPRPPGSLAKHP SVSADEELSATLARDIVLVSAILFFLLALRIPQRLCQRLRIRLP HRYQRLRTED	HCMV protein UL16
29	MCRRESLRTLPLFWVLLSCPRLLEYSSSSFPFATADIAEKMW AENYETTSPAPVLVAEGEQVTIPCTVMTHSWPMVSIRARFCRS HDGSDELILDAVKGHRLMNGLQYRLPYATWNFSQLHLGQIFSL TFNVSTDTAGMYECVLRNYSHGLIMQRFVILTQLETLSRPDEPC CTPALGRYSLGDQIWSPTPWRLRNHDCGMYRGFQRNYFYIGR ADAEDCWKPACPDEEPPDRCWTVIQRYRLPGDCYRSQPHPPKFL PVTAPPADIDTGMSPWATRGI AAF LGFWSIFTVCFLCYLCYLQ CCGRWCPTPGRGRRGEGGYRRLPTYDSYPGVKKMKR	HCMV protein UL141
30	MRIEWACWLFGYFVSSVGSERSLSYRYHLESNSSANVVCNGNI SVFVNGTLGVRYNITVGISSSLLIGHTIQTLESWFTPWVQNKSY SKQPLSTTETLYNIDSENIHRVSQYFHTRWIKSLQENHTCDLTNS TPTYTYQANVNNTNYLTLTSSGWQDRLNYTAINSTHFNLTESNI TSIHKYLN TTCIERLRNYTLEPVYTTAVPQNVTP EHAITTL YTP PNAITIKDTTQSHTVQTPSFNDTHNVTEHTLNISYVLSQKTNNTT SPWVYAIPMGATATIGAGLYIGKHFTPVKFVYEVWRGQ	HCMV protein UL142
31	MGGKWSKSKTAGWPEVRERIRNAPSAAAPGVGAVSQDLAKH GAITSSNANHPSCVWLEAQEDEEVGFVVRPQVPLRPMTYKGAL DLSHFLKEKGGLEGLIYSRRRQEILDLWVYHTQGYFPDWQNYT	HIV Nef

	PGPGIRYPLTFGWCFKLVPVEPEEVEKATEGENNSLLHPICQHG MDDEEGEVLKWQFDPRLALKHRAQELHPEFYKDC	
32	QPIPIVAIVALVVAIIAIVVWSIVIIIEYRKILRQRKIDRLIDRLIER AEDSGNESEGEISALVEMGVEMGHHAPWDVDDL	HIV Vpu
33	MWTILLFCVPVIYGELYPDFCPLAVVDFDVNATVDDLLFDISL SKQCSDDKIRHSAVAAMTDNAFFFGNSETQIETDFGKYLAFC YQVFSTLNHFLFKNFKKTKGLMKRYDKLCLDVESYIHIQICS KSFIRLRMRNETGISPRILETTFYLQNKRNSTWVAIKNYLGEDD PFTYRIWHTLTHAKNFLINSCENDFNQLFFWQRKYLSLAKT FEATFKQGFNPMIEQRNEQRYRTNNIDCSFSKFRQNGVKVAVCKYT GWGVSGFGSLEVLQKIKSPFGEEWKRVGFNSTGAFTPLYGSDV LWGLIFLRVEMTTYVCTCTNKNTGTQIQVTLPDVDLDDLSEK TSSNVFVDMLCYTLIAILFLAFVTAVVLLGVSCLDGVQKVLTW PLQHIQKEPVSEKIINLTNLMFGQEPLPKKESLKQQCL	HHV-7 U21
34	MEDEDVPVCWICNEELGNERFRACGCTGELENVHRSCSLSTWLT ISRNTACQICGVVYNTRVVWRPLREMTLLPRLTYQEGLELIVFI FIMTLGAAGLAAATWVWLYIVGGHDPEIDHVA AAAAYYVFFVF YQLFVVFGGLGAFFHMMRHVGRAYAAVNTRVEVFPYRPRPTSP ECAVEEIELQEILPRGDNQDEEGPAGAAPGDQNGPAGAAPGDQ DGPADGAPVHRDSEESVDEAAGYKEAGEPTHNDGRDDNVEPT AVGCDCNNLGAERYRATYCGGYVGAQSGDGAYSVSCHNKAG PSSLVDILPQGLPGGGYGSMGVIRKRSVSSALMFH	HHV-8 KK3
35	MASKDVEEGVEGPICWICREEVGNEGIHPCACTGELD VVHPQC LSTWLT VSRNTACQMCRVIYRTRTQWRSRLNLWPEMERQEIFE LFLMSVVVAGLVGVALCTWTLLVILTAPAGTFSPGAVLGFLC FFGFYQIFIVFAFGGICRVSGTVRALYAANNTRVTVLPYRRPRR PTANEDNIELTVLVGPAGGTDEEPTDESSEGDVASGDKERDGSS GDEPDGGPNDRAGLRGTARTDLCAPT KKPVRKNHPKNNG	HHV-8 KK5
36	MDSTGEFCWICHQPEGPLKRFCGCKGSCAVSHQDCLRGWLETS RRQTCALCGTPYSMKWKTKPLREWTWGEEVLAAMEACLPL VLIPLAVLMIVMGTWLLVNHNGFLSPRMQVVLVIVLLAMIVF SASASYVMVEGPGCLDTCTAKNSTVTVNSIDEAIATQQPTKTD LGLARETLSTRFRRGKCRSCCRLGCVRLCCV	MHV-68 MK3

37	MLFRLLSPLSPLALTALLLFLPPSDVSGLLLRPPPAPCLLLFLPF QILSGLLFLLFLPLFFSLPLLLSPSLPITMRFPARWRFLPWKAPSQ PAAAF LF	HTLV-1 p12
38	MKIKLSIIRWLTLLALTIFRMRS LVIVLLFPSIIYSMVIRRCEKME EETWKLKIGMCIQAKDFYSKRTDCSVHRSDVGGGLITEGNGYR VVVHDQCEEPNPFIIATTKQTHFGVTHSYIEFSNSNTGAPENIPD CSKHILISVYCDQEASGLDFHTLKYVESNYLHITVKYDTSCINH LGVNYSFMNECERKLKSIYETDTLTGAKDTQTRDKYLKTCTN TKFDRSVYKTHMQKSKILHVKTEL	CPXV203
39	MRYMILGLLALAAVCSAAKKVEFKPACNVTFKSEANECTTLI KCTTEHEKLIIRHKDKIGKYAVYAIWQPGDTNDYNVTVFQGEN RKTFMYKFPFYEMCDITMYMSKQYKLWPPQKCLENTGTFCST ALLITALALVCTLLYLKYKSRRSFIDEKKMP	E3-19K (HAdV-2)
40	MGAILVVLALLSLLGLGSANLNPLDHDPCLD FDPENCTLT FAPD TSRLCGVLIKCGWDCRSVEITHNNKTWNNTLSTTWEPGVPQW YTVSVRGPDG SIRISNNTFIFSEMCDLAMFMSRQYDLWPPSKEN IVAFSIA YCLVTCIITAICVCIHLLIVIRPRQSNEEKEKMP	E3-19K (HAdV-3)
41	MIRYIILGLLTLASA HGTTQKVDFKEPACNVTFAAEANECTTLI KCTTEHEKLLIRHKNKIGKYAVYAIWQPGDTTEYNVTVFQGKS HKTFMYTFPFYEMCDITMYMSKQYKLWPPQNCVENTGTFCCT AMLITVLALVCTLLYIKYKSRRSFIEEKKMP	E3-19K (HAdV-5)
42	MRYMILGLLALAAVCSAAKKVEFKPACNVTFKSEANECTTLI KCTTEHEKLIIRHKDKIGKYAVYAIWQPGDTNDYNVTVFQGEN RKTFMYKFPFYEMCDITMYMSKQYKLWPPQKCLENTGTFCST ALLITALALVCTLLYLKYKSRRSFIDEKKMP	E3-19K (HAdV-6)
43	MGPI LVLLVLLSLLEPGSANYDPCLDFDPENCTLT FAPDTSRICG VLIKCGWE CRSVEITHNNKTWNNTLSTTWEPGVPEWYTVSVR GPDGSIRISNNTFIFSEMCDLAMFMSKQYSLWPPSKDNIVTFSIA YCLCACLLTALLCVCIHLLVTTRIKNANNKEKMP	E3-19K (HAdV-11)
44	MGPI LVLLVLLSLLEPGSANYDPCLDFDPENCTLT FAPDTSRICG VLIKCGWE CRSVEITHNNKTWNNTLSTTWEPGVPEWYTVSVR GPDGSIRISNNTFIFSEMCDLAMFMSKQYSLWPPSKDNIVTFSIA YCLCACLLTALLCVCIHLLVTTRIKNANNKEKMP	E3-19K (HAdV-35)

45	MSSTLPALLCVGLCLSQRISAQQQTLPKPFIWAEPHFMVPKEKQ VTICCCQGNYGAVEYQLHFEGSLFAVDRPKPPERINKVKFYIPDM NSRMAGQYSCIYRVGELWSEPSNLLDLVVTEMYDTPTLSVHPG PEVISGEKVTFYCRLDTATSMFLLLKEGRSSHVQRGYGVQAE FPLGPVTTAHRGTYRCFGSYNNHAWSPSEPVKLLVTGDIENTS LAPEDPTFPADTWGTYLLTTETGLQKDHAWDHTAQNLLRMG LAFLVLVALVWFLVEDWLSRKRTRERASRASTWEGRRRLNTQ TL	NCR1(Nkp46)
46	MAWRALHPLLLLLLLFPGSQAQSKAQVLQSVAGQTLTVRC QYPPTGSLYEKKGWCKEASALVCIRLVTSSKPRTMAWTSR FTIWDDPDAGFFVTMTDLREEDSGHYWCRIYRPSDNSVSK SVRFYLVVSPASASTQTSWTPRDLVSSQTQTQSCVPPTAGAR QAPESPSTIPVPSQPQNSTLRPGPAAPIALVPVFCGLLVAKSLV LSALLVWWGDIWWKTMMELRSLDTQKATCHLQQVTDLPWTS VSSPVEREILYHTVARTKISDDDDDEHTL	NCR2(Nkp44)
47	MAWMLLLILIMVHPGSCALWVSQPPEIRTLEGSSAFLPCSFNAS QGRLAIGSVTWFRDEVVPGKEVRNGTPEFRGRLAPLASSRFLH DHQAELHIRDVRGHDASIYVCRVEVLGLGVGTGNGTRLVVEK EHPQLGAGTVLLLRAGFYAVSFLSVAVGSTVYYYQGKCLTWKG PRRQLPAVVPAPLPPPCGSSAHLPPVPGG	NCR3 (Nkp30)
48	MAWRALHPLLLLLLLFPGSQAQSKAQVLQSVAGQTLTVRC QYPPTGSLYEKKGWCKEASALVCIRLVTSSKPRTMAWTSR FTIWDDPDAGFFVTMTDLREEDSGHYWCRIYRPSDNSVSK SVRFYLVVSPASASTQTSWTPRDLVSSQTQTQSCVPPTAGAR QAPESPSTIPVPSQPQNSTLRPGPAAPIAFWVLVVVGGVLACY SLLVTVAFIIFWVRSKRSRGHSDYMNMTPRRPGPTRKHYPY APPRDFAAYRSRVKFSRSADAPAYQOGQNQLYNELNLGRREE <u>YDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQDKMAEAYS</u> <u>EIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR</u>	NCR2 ED- CD28 TM- CD28 ICD- CD3Z ICD

Claims

1. A genetically modified mammalian immune cell or a population thereof, comprising a surface molecule and a protection element,

wherein the surface molecule is expressed on the surface of the immune cell, and is capable of binding to one or more target NK receptor ligand (tNKRL) expressed on a target cell thereby activating the immune cell to exert cytotoxic effect on the target cell,

wherein the immune cell, when activated, is capable of being induced to express one or more induced NK receptor ligand (iNKRL) that is capable of binding to the surface molecule, and

wherein the protection element substantially suppresses expression of the one or more induced NK receptor ligand on the immune cell surface following activation of the immune cell, thereby preventing the activated immune cell from becoming a target cell;

preferably, wherein at least one of the target NK receptor ligands is identical to at least one of the induced NK receptor ligands, or wherein the surface molecule cross-reacts with at least one of the target NK receptor ligands and at least one of the induced NK receptor ligands;

preferably, wherein the one or more target NK receptor ligand is selected from the group consisting of: NKG2D ligands, NCR ligands, KIR ligands, CD226 ligands, TIGIT ligands, CD96 ligands, or any combination thereof;

more preferably, the one or more target NK receptor ligand comprises one or more NKG2D ligand;

more preferably, the NKG2D ligand is selected from the group consisting of: MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6.

2. The genetically modified mammalian immune cell of claim 1, wherein the expression of the one or more induced NK receptor ligand on the immune cell surface is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%) relative to expression of the one or more induced NK receptor ligand on a reference immune cell surface without the protection element;
optionally, wherein the expression of the one or more induced NK receptor ligand on the immune cell surface is reduced such that the binding the one or more induced NK receptor

ligand to the surface molecule is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%);

optionally, wherein the expression of the one or more induced NK receptor ligand is detectable on no more than 50% (e.g., no more than 40%, no more than 30%, no more than 20%, or no more than 10%) of the population of the immune cells following binding of the surface molecule to the one or more target NK receptor ligand;

optionally, wherein the one or more induced NK receptor ligand comprises MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6 and the expression level of the MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and/or ULBP6 on the immune cell surface is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%);

optionally, wherein the one or more induced NK receptor ligand comprises MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP5 and ULBP6 and the expression level of each of the MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP5 and ULBP6 on the immune cell surface is reduced by at least 10% (e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%).

3. The genetically modified mammalian immune cell of claim 1 or 2, wherein the immune cell has substantially reduced activation-induced cell death of the immune cell, optionally the reduced activation-induced cell death of the immune cell can be determined by reduced apoptosis detected by Annexin V/7-AAD;

optionally, wherein the immune cell is capable of proliferate upon activation (e.g., having an increased proliferation rate as compared to a modified mammalian immune cell without the protection element).

4. The genetically modified mammalian immune cell of any one of claims 1 to 3, wherein the protection element is capable of silencing expression of the one or more induced NK receptor ligand or is capable of inducing intracellular retention and/or degradation of the one or more induced NK receptor ligand;

optionally, the protection element comprises a small RNA (e.g., an siRNA or miRNA or sh RNA) that targets mRNAs of the one or more induced NK receptor ligand;

optionally, wherein the protection element comprises a small RNA, such as HCMV encoded microRNAs (miR-20a, miR-UL112) are able to modulate NKG2D-ligand expression.

5. The genetically modified mammalian immune cell of claim 4, the protection element comprises one or more protein degradation element that is capable of inducing intracellular retention and/or degradation of the one or more induced NK receptor ligand;

optionally, wherein at least one of (or all of) the protein degradation elements comprise an endoplasmic reticulum (ER)-retention domain and an iNKRL binding domain capable of binding to the one or more induced NK receptor ligand;

optionally, wherein the protein degradation element further comprises an ER-retention transmembrane domain, wherein the ER-retention transmembrane domain has one end operably linked to the iNKRL binding domain and the other end operably linked to the ER retention domain;

optionally, wherein the ER-retention domain and/or the ER-retention transmembrane domain is derived from an ER-retention virus protein;

optionally, wherein the ER-retention virus protein is selected from the group consisting of: HCMV protein US2, HCMV protein US11, HCMV protein US18, HCMV protein US20, HCMV protein US3, HCMV protein US10, HCMV protein US6, HSV ICP47, CPXV12, BHV UL49.5, EBV BNFL2a, HCMV protein UL16, HCMV protein UL141, HCMV protein UL142, HIV Nef, HIV Vpu, HHV-7 U21, HHV-8 KK3, HHV-8 KK5, MHV-68 MK3, HTLV-1 p12, Cowpox Virusprotein CPXV203 and Ad E3/19K;

optionally, wherein the ER-retention domain and the ER-retention transmembrane domain are both derived from the same ER-retention virus protein;

optionally, wherein the iNKRL binding domain comprises an antibody domain (e.g., scFv) capable of binding to the one or more induced NK receptor ligand;

optionally, wherein the iNKRL binding domain is derived from a virus protein capable of binding to the one or more induced NK receptor ligand;

optionally, wherein the one or more induced NK receptor ligand comprises one or more NKG2D ligands;

optionally, wherein the one or more NKG2D ligands are selected from the group consisting of: MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6;

optionally, wherein the virus protein capable of binding to the one or more NK receptor ligand is derived from a virus selected from the group consisting of: adenovirus (Ad) (e.g., human Ad), cytomegalovirus (CMV) (human CMV, i.e., HCMV) and Kaposi's sarcoma-associated herpesvirus (KSHV);

optionally, wherein the virus protein capable of binding to the one or more NK receptor ligand is derived from a virus protein selected from the group consisting of: Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), HCMV protein UL16, HCMV protein UL142, HCMV protein US18, HCMV protein US20, and KSHV K5;

optionally, wherein the ER-retention domain and the binding domain are both derived from the same virus protein;

optionally, wherein the ER-retention domain, the ER-retention transmembrane domain and the binding domain are all derived from different virus protein;

optionally, wherein the same virus protein is selected from the group consisting of Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), HCMV protein UL16, HCMV protein UL142, HCMV protein US18, HCMV protein US20, KSHV K5;

optionally, wherein the protein degradation element comprises a virus protein selected from the group consisting of: Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), HCMV protein UL16, HCMV protein UL142, HCMV protein US18, HCMV protein US20, KSHV K5 or an analog thereof, or a derivative thereof;

optionally, wherein the one or more protein degradation elements comprise a combination of virus proteins, wherein the combination of virus proteins comprises a HCMV protein UL16 and another virus protein selected from the group consisting of: Ad E3/19K (e.g., Ad2 E3/19K, Ad5 E3/19K), HCMV protein UL142, HCMV protein US18, HCMV protein US20, and KSHV K5.

6. The genetically modified mammalian immune cell of claim 5, wherein the one or more protein degradation elements further comprise a protein degradation pathway (PDP) binding domain capable of binding to a member in a protein degradation pathway;
optionally, wherein the PDP binding domain is operably linked to the ER-retention domain;
optionally, wherein the protein degradation pathway is ubiquitylation-proteosome pathway, endosome-lysosome pathway, or autophagic degradation pathway;

optionally, wherein the member in the ubiquitylation-proteosome pathway comprises E1 ubiquitin activating enzyme, E2 ubiquitin conjugating enzyme, or E3 ubiquitin ligase;

optionally, wherein the member in the endosome-lysosome pathway comprises AP-1, AP-2, AP-3, endosome, lysosome, HOPS, ESCRT, GASP, BLOC-1, ESCRT, Retromer, ESCRT, sortingnexin, Dapper2, SNX4, Pincher, Rap1-PDZ-GEF1, clathrin, or C3G/CrkL/Shp2/Gab2;

optionally, wherein the member in the autophagic degradation pathway comprises chaperone-mediated autophagy (CMA) USP10, G3BP1, ULK1, ATG16L1, TRIM16, FBXO27VDAC, RHOT1, MFN1/2, BNIP3L, FUNDC1, BNIP3, AMBRA1, BCL2LI3, FKBP8, CHDH, DISC1, PHB2, Cardiolipin, SEC62, RTN3, PEX5, PEX14, ABCD3, or NUFIP1.

7. The genetically modified mammalian immune cell of any one of claims 1 to 6, wherein the surface molecule comprises a) a tNKRL-binding extracellular domain capable of binding to the one or more target NK receptor ligand, and b) a surface molecule (SM) intracellular domain capable of activating the immune cell following binding of the tNKRL-binding extracellular domain to the one or more target NK receptor ligand, and wherein the tNKRL-binding extracellular domain is associated with the SM intracellular domain to allow transduction of binding signal from the tNKRL-binding extracellular domain to the SM intracellular domain;

optionally, wherein the tNKRL-binding extracellular domain comprises an antibody domain that is capable of binding to the one or more target NK receptor ligand;

optionally, wherein the tNKRL-binding extracellular domain comprises an NK receptor extracellular domain that is capable of binding to the one or more target NK receptor ligand;

optionally, wherein the tNKRL-binding extracellular domain comprises an NKG2D extracellular domain;

optionally, wherein the receptor further comprises a SM transmembrane domain, wherein the SM transmembrane domain has one end operably linked to the tNKRL-binding extracellular domain and the other end operably linked to the SM intracellular domain;

optionally, wherein the SM transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of CD3, CD4, CD8, CD28, B7, ICOS, CD226, 41-BB, OX40, CD27, GITR, HVEM, CD40, BAFFR, BAFF, NKG2D, NKG2C, NKG2A, NKp44, NKp46, NKp30.

8. The genetically modified mammalian immune cell of any one of claims 1 to 7, wherein the surface molecule comprises a tNKRL-binding chimeric antigen receptor (CAR);
optionally, wherein the tNKRL-binding chimeric antigen receptor (CAR) comprises an CNK receptor protein complex comprising a first polypeptide comprising the tNKRL-binding extracellular domain operably linked to a first CNK transmembrane domain, and a second polypeptide comprising the CNK intracellular domain operably linked to a second CNK transmembrane domain, wherein the first CNK transmembrane domain and the second CNK transmembrane domain associate with each other to form the protein complex;
optionally, wherein the first CNK transmembrane domain comprises a NK receptor transmembrane domain, optionally an NKG2D transmembrane domain;
optionally, wherein the first transmembrane domain is capable of forming a first dimer;
optionally, wherein the first polypeptide comprises a NKG2D protein;
optionally, wherein the NKG2D protein comprises the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2;
optionally, wherein the second transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of DNAX-Activating Protein of 10 kDa (DAP10) and DNAX-Activating Protein of 12 kDa (DAP12);
optionally, wherein the CNK intracellular domain comprises a cytoplasmic domain of a protein selected from the group consisting of: 4-1BB (CD137), CD2, CD3, CD35, CD25, CD27, CD28, CD30, CD40, CD79A, CD79B, CARD11, DAP10, DAP12, Fc receptor, Fyn, LIGHT, LT β R, HVEM, ICOS, Lck, LAG3, LAT, LRP, NOTCH1, NOTCH2, NOTCH3, NOTCH4, OX40 (CD134), ROR2, Ryk, SLAMF1, Slp76, pTa, TCR α , vTCRP, TRIM, Zap70, PTCH2, IL7 receptor, IL15 receptor and GITR;
optionally, wherein the CNK intracellular domain comprises a cytoplasmic domain of CD3 (e.g., CD3 zeta);
optionally, wherein the CNK intracellular domain comprises the amino acid sequence of SEQ ID NO: 5.
9. The genetically modified mammalian immune cell of claim 8, wherein the second polypeptide comprises a chimeric adaptor protein comprising:

- a) the transmembrane domain of DAP10 operably linked to the intracellular domain of CD3 (e.g., CD3 zeta);
- b) the transmembrane domain of DAP12 operably linked to the intracellular domain of CD3 (e.g., CD3 zeta); or
- c) the transmembrane domain of DAP10 operably linked to the intracellular domain of DAP12;

optionally, wherein the second polypeptide comprises a full-length DAP10 operably linked to the intracellular domain of CD3 zeta;

optionally, wherein the chimeric adaptor protein comprises the amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 16;

optionally, wherein the second CNK transmembrane domain is capable of forming a second dimer, and wherein the second dimer associates with the first CNK transmembrane domain to form the complex;

optionally, wherein the CNK receptor comprises a hexameric protein complex comprising a homodimer of the NKG2D protein and two homodimers of the chimeric adaptor protein.

10. The genetically modified mammalian immune cell of any one of the preceding claims, wherein the immune cell is further modified to be deficient in a) MHC class I protein, or b) MHC class II protein or MHC class II transactivator, or c) both a) and b);

optionally, wherein the immune cell is T cell and the immune cell is further modified to be deficient in endogenous TCR.

11. The genetically modified mammalian immune cell of any one of claims 1 to 10, further expressing a target binding (TB) receptor capable of binding to a target marker;

optionally, wherein the target marker is expressed on the target cell;

optionally, wherein the TB receptor comprises a target-marker binding extracellular domain capable of binding to the target marker and a TB intracellular domain capable of activating the immune cell upon binding of the target-marker binding extracellular domain to the target marker;

optionally, wherein the TB receptor further comprises a TB transmembrane domain;

optionally, wherein the TB transmembrane domain has one end operably linked to the target-marker binding extracellular domain and the other end operably linked to the TB intracellular domain;

optionally, wherein the target-marker binding extracellular domain comprises the extracellular domain of a natural cytotoxicity receptor (e.g., NKp30 (NCR3), NKp44 (NCR2), and NKp46 (NCR1));

optionally, wherein the TB transmembrane domain comprises the transmembrane domain of a protein selected from the group consisting of CD3, CD4, CD8, CD28, B7, ICOS, CD226, 41-BB, OX40, CD27, GITR, HVEM, CD40, BAFFR, BAFF, NKG2D, NKG2C, NKG2A, NKp44, NKp46, NKp30;

optionally, wherein the TB intracellular domain comprises a cytoplasmic domain of a protein selected from the group consisting of: 4-1BB (CD137), CD2, CD3, CD35, CD25, CD27, CD28, CD30, CD40, CD79A, CD79B, CARD11, DAP10, DAP12, Fc receptor, Fyn, LIGHT, LT β R, HVEM, ICOS, Lck, LAG3, LAT, LRP, NOTCH1, NOTCH2, NOTCH3, NOTCH4, OX40 (CD134), ROR2, Ryk, SLAMF1, Slp76, pTa, TCR α , vTCRP, TRIM, Zap70, PTCH2, IL7 receptor, IL15 receptor and GITR;

optionally, wherein the TB intracellular domain comprises a cytoplasmic domain of CD28 operably linked to CD3 zeta chain;

optionally, wherein the target marker is a tumor antigen, an auto-reactive cell antigen or a virus antigen, bacteria antigen, a microbe-infected cell, or damaged and/or aged cells;

optionally, wherein the target cell is a tumor cell, an auto-reactive cell, a senescent cell or a virus-infected cell.

12. The genetically modified mammalian immune cell of any one of the preceding claims, wherein the immune cell is a T cell, a Natural Killer (NK) cell, a NKT cell, a B cell, a hematopoietic cell (e.g., bone marrow cell), a thymocyte, a dendritic cell (e.g., a mature dendritic cell), a macrophage cell, a tumor infiltrating lymphocyte, a monocyte, a granulocyte;

optionally, wherein the immune cell is a T cell, such as CD4⁺ T cell, CD8⁺ T cell, cytotoxic T cell, terminal effector T cell, memory T cell, na ν e T cell, natural killer T cell, gamma-delta T cell, cytokine-induced killer (CIK) T cell, or tumor infiltrating lymphocyte.
13. A pharmaceutical composition comprising the genetically modified mammalian immune cell or any one of claims 1 to 12, or the population thereof.
14. A method for treating a disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 13;

optionally, wherein the disease is cancer, a disease associated with auto-reactive lymphocytes (e.g., an autoimmune disease), an immune deficient disease, or an aging associated disease;

optionally, wherein the autoimmune disease is organ-specific autoimmune disease or systemic autoimmune disease;

optionally, wherein the organ-specific autoimmune disease is selected from the group consisting of chronic lymphocytic thyroiditis, hyperthyroidism, insulin-dependent diabetes mellitus, myasthenia gravis, ulcerative colitis, pernicious anemia with chronic atrophic gastritis, pulmonary hemorrhage nephritic syndrome, pemphigus vulgaris, pemphigoid, primary biliary cirrhosis, multiple sclerosis, acute idiopathic polyneuritis, etc;

optionally, wherein the systemic autoimmune disease is selected from the group consisting of systemic lupus erythematosus, rheumatoid arthritis, systemic vasculitis, scleroderma, pemphigus, dermatomyositis, mixed connective tissue disease, autoimmune hemolytic anemia, thyroid autoimmune disease, and ulcerative colitis;

optionally, wherein the autoimmune disease is Graft versus host disease (GvHD).

15. An expression construct encoding a surface molecule and a protection element, wherein the construct can be expressed in an immune cell, wherein the surface molecule is as defined in any one of claims 1 to 12, and the protection element is as defined in any one of the claims 1 to 12.
16. A method of producing the genetically modified mammalian immune cell of any one of the claims 1 to 12, comprising introducing the expression construct of claim 15 to a starting immune cell under conditions to allow expression of the surface molecule on the surface of the immune cell and to allow expression of the protection element in the immune cell.