in which allogeneic therapeutic cells are pre-manufactured, characterized in detail, and available for immediate administration to a broad range of patients.

[0004] Allogeneic immunotherapy remains a dangerous procedure with many possible complications, such as allogeneic T-cell responses, which is clinically manifested as graft-versus-host disease (GVHD) and/or Host-versus-Graft disease (HvGD, graft rejection). GvHD is caused by the attack of recipient tissues by infused allogeneic CAR-T cell mediated by alloreactive TCR on donor CAR cells. In particular, endogenous T-cell receptor alpha (TCRα; TRAC) and beta (TCRβ; TRBC) chains on infused T cells may recognize major and minor histocompatibility antigens in the recipient, leading to (GvHD). Conversely, infused allogeneic CART cells may be rejected by the recipient T lymphocytes leading to HvGD. As such, the applicability and versatility of adoptive immunotherapy will be improved by the use of allogeneic CAR T cells if and when a standardized solution that controls inherent allogeneic immune responses is identified. The specific inhibition of GvHD will enable the safe and effective use of allogeneic CART-cells.

[0005] Accordingly, a need exists for improved methods of CAR-T cells that does not invoke a host immune response. Specifically, there is a need for novel and alternative compositions and methods for generating allogeneic T cells with improved fitness.

[0006] The present invention provides methods and compositions that address these needs.

SUMMARY

[0007] In one example, the present disclosure provides a modified immune cell comprising: (a) an insertion and/or deletion in one or more gene loci, each encoding an endogenous immune protein selected from the group consisting of CD3δ, CD3ε, CD3γ, B2M, CIITA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, and invariant chain (Ii Chain). The insertion and/or deletion is capable of downregulating gene expression of the one or more endogenous immune genes. In addition, the modified immune cell comprises (b) an exogenous nucleic acid encoding a chimeric antigen receptor (CAR), an engineered T cell receptor (TCR), a Killer cell immunoglobulin-like receptor (KIR), an antigen-binding polypeptide, a cell surface receptor ligand, or a tumor antigen. In some examples, the modified immune cell further comprises a dominant negative receptor, a switch receptor, a chemokine, a chemokine receptor, a cytokine, a cytokine receptor, IL-7, IL-7R, IL-15, IL-15R, IL-21, IL-18, CCL21, CCL19, or any

combination thereof. In one aspect, the present disclosure refers to a modified immune cell comprising: (a) an insertion and/or deletion in one or more gene loci each encoding an endogenous immune protein selected from the group consisting of CD3δ, CD3ε, CD3γ, B2M, CIITA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, and invariant chain (Ii Chain), wherein the insertion and/or deletion is capable of downregulating gene expression of the one or more endogenous immune genes; and (b) an exogenous nucleic acid encoding a chimeric antigen receptor (CAR), an engineered T cell receptor (TCR), a Killer cell immunoglobulin-like receptor (KIR), an antigen-binding polypeptide, a cell surface receptor ligand, or a tumor antigen; and optionally (c) further comprising a dominant negative receptor, a switch receptor, a chemokine, a chemokine receptor, a cytokine, a cytokine receptor, IL-7, IL-7R, IL-15, IL-15R, IL-21, IL-18, CCL21, CCL19, or a combination thereof.

[0008] In some examples, the insertion and/or deletion is capable of downregulating the gene expression of: (a) a T cell receptor subunit selected from CD3δ, CD3ε, and/or CD3γ; (b) a HLA class I molecule selected from B2M, TAP1, TAP2, TAPBP, and/or NLRC5; and (c) a HLA class II molecule selected from HLA-DM, RFX5, RFXANK, RFXAP, and/or invariant chain (Ii Chain).

[0009] In some examples, the insertion and/or deletion is capable of downregulating (a) the gene expression of CD3δ, and (b) the gene expression of a HLA molecule selected from the group consisting of B2M, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, invariant chain (Ii Chain), and any combination thereof.

[0010] In some examples, the insertion and/or deletion is capable of downregulating: (a) the gene expression of CD3ε, and (b) the gene expression of a HLA molecule selected from the group consisting of B2M, TAP1, TAP2, TAPBP, NLRC5, CIITA, HLA-DM, RFX5, RFXANK, RFXAP, invariant chain (Ii Chain), or any combination thereof.

[0011] In some examples, the insertion and/or deletion is capable of downregulating: (a) the gene expression of CD3γ, and (b) the gene expression of a HLA molecule selected from the group consisting of B2M, TAP1, TAP2, TAPBP, NLRC5, CIITA, HLA-DM, RFX5, RFXANK, RFXAP, invariant chain (Ii Chain), or any combination thereof.

[0012] In some examples, the insertion and/or deletion is capable of downregulating the gene expression of: (a) CD3ε, B2M, and CIITA; (b) CD3ε, B2M, and RFX5; (c) CD3ε, B2M, and RFXAP; (d) CD3ε, B2M, and RFXANK; (e) CD3ε, B2M, and HLA-DM; (f) CD3ε, B2M, and Ii chain; (g) CD3ε, TAP1, and CIITA; (h) CD3ε, TAP1, and RFX5; (i) CD3ε, TAP1, and RFXAP; (j) CD3ε, TAP1, and RFXANK; (k) CD3ε, TAP1, and HLA-DM; (l) CD3ε, TAP1, and Ii chain; (m) CD3ε, TAP2, and CIITA; (n) CD3ε, TAP2, and RFX5; (o) CD3ε, TAP2, and RFXAP; (p) CD3ε, TAP2, and RFXANK; (q) CD3ε, TAP2, and HLA-DM; (r) CD3ε, TAP2, and Ii chain; (s) CD3ε, NLRC5, and CIITA; (t) CD3ε, NLRC5, and RFX5; (u) CD3ε, NLRC5, and RFXAP; (v) CD3ε, NLRC5, and RFXANK; (w) CD3ε, NLRC5, and HLA-DM; (x) CD3ε, NLRC5, and Ii chain; (y) CD3ε, TAPBP, and CIITA; (z) CD3ε, TAPBP, and RFX5; (aa) CD3ε, TAPBP, and RFXAP; (bb) CD3ε, TAPBP, and RFXANK; (cc) CD3ε, TAPBP, and HLA-DM; or (dd) CD3ε, TAPBP, and Ii chain.

[0013] In some examples, the insertion and/or deletion is capable of downregulating the gene expression of: (a) CD3δ, B2M, and CIITA; (b) CD3δ, B2M, and RFX5; (c) CD3δ, B2M, and RFXAP; (d) CD3δ, B2M, and RFXANK; (e) CD3δ, B2M, and HLA-DM; (f) CD3δ, B2M, and Ii chain; (g) CD3δ, TAP1, and CIITA; (h) CD3δ, TAP1, and RFX5; (i) CD3δ, TAP1, and RFXAP; (j) CD3δ, TAP1, and RFXANK; (k) CD3δ, TAP1, and HLA-DM; (l) CD3δ, TAP1, and Ii chain; (m) CD3δ, TAP2, and CIITA; (n) CD3δ, TAP2, and RFX5; (o) CD3δ, TAP2, and RFXAP; CD3δ, TAP2, and RFXANK; CD3δ, TAP2, and HLA-DM; (r) CD3δ, TAP2, and Ii chain; (s) CD3δ, NLRC5, and CIITA; (t) CD3δ, NLRC5, and RFX5; (u) CD3δ, NLRC5, and RFXAP; (v) CD3δ, NLRC5, and RFXANK; (w) CD3δ, NLRC5, and HLA-DM; (x) CD3δ, NLRC5, and Ii chain; (y) CD3δ, TAPBP, and CIITA; (z) CD3δ, TAPBP, and RFX5; (aa) CD3δ, TAPBP, and RFXAP; (bb) CD3δ, TAPBP, and RFXANK; (cc) CD3δ, TAPBP, and HLA-DM; or (dd) CD3δ, TAPBP, and Ii chain.

[0014] In some examples, the insertion and/or deletion is capable of downregulating the gene expression of: (a) CD3γ, B2M, and CIITA; (b) CD3γ, B2M, and RFX5; (c) CD3γ, B2M, and RFXAP; (d) CD3γ, B2M, and RFXANK; (e) CD3γ, B2M, and HLA-DM; (f) CD3γ, B2M, and Ii chain; (g) CD3γ, TAP1, and CIITA; (h) CD3γ, TAP1, and RFX5; (i) CD3γ, TAP1, and RFXAP; (j) CD3γ, TAP1, and RFXANK; (k) CD3γ, TAP1, and HLA-DM; (l) CD3γ, TAP1, and Ii chain; (m) CD3γ, TAP2, and CIITA; (n) CD3γ, TAP2, and RFX5; (o) CD3γ, TAP2, and RFXAP; (p) CD3γ, TAP2, and RFXANK; (q) CD3γ, TAP2, and HLA-DM; (r) CD3γ, TAP2, and Ii chain; (s) CD3γ, NLRC5, and CIITA; (t) CD3γ, NLRC5, and RFX5; (u) CD3γ, NLRC5, and RFXAP; (v) CD3γ, NLRC5, and RFXANK; (w) CD3γ, NLRC5, and HLA-DM; (x) CD3γ, NLRC5, and Ii chain; (y) CD3γ, TAPBP, and CIITA; (z) CD3γ, TAPBP, and RFX5; (aa) CD3γ, TAPBP, and RFXAP; (bb) CD3γ, TAPBP, and RFXANK; (cc) CD3γ, TAPBP, and HLA-DM; or (dd) CD3γ, TAPBP, and Ii chain.

[0015] In some examples, the modified immune cell is selected from the group consisting of a T cell, a natural killer cell (NK cell), a natural killer T cell, a lymphoid progenitor cell, a hematopoietic stem cell, a stem cell, a macrophage, a dendritic cell, or any combination thereof. In some examples, the modified immune cell is a CD4+ T cell or a CD8+ T cell. In some examples, the modified immune cell is an allogeneic T cell or autologous human T cell.

[0016] In some examples, the insertion and/or deletion is the result of gene editing selected from the group consisting of: (a) a CRISPR-associated (Cas) (CRISPR-Cas) endonuclease system and a guide RNA; (b) a TALEN gene editing system, a zinc finger nuclease (ZFN) gene editing system, a meganuclease gene editing system, or a mega-TALEN gene editing system; and (c) a gene silencing system selected from antisense RNA, antigomer RNA, RNAi, siRNA, or shRNA. In some examples, the CRISPR-Cas system comprises a pAd5/F35-CRISPR vector. In some examples, the Cas endonuclease comprises Cas3, Cas4, Cas8a, Cas8b, Cas9, Cas10, Cas10d, Cas12a, Cas12b, Cas12d, Cas12e, Cas12f, Cas12g, Cas12h, Cas12i, Cas13, Cas14, CasX, Cse1, Csy1, Csn2, Cpf1, C2c1, Csm2, Cmr5, Fok1, *S. pyogenes* Cas9, *Staphylococcus aureus* Cas9, MAD7 nuclease (a type V CRISPR nuclease), or any combination thereof.

[0017] In some examples, the CRISPR-Cas endonuclease system comprises a guide RNA. In some examples, the guide RNA comprises a guide sequence that is complementary with a sequence within the one or more gene loci each encoding the immune protein selected from the group consisting of CD3δ, CD3ε, CD3γ, B2M, CIITA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, and invariant chain (Ii Chain). In some examples, the guide RNA is complementary with a sequence within one or more exons of CD3δ, CD3ε, or CD3γ. In some examples, the guide RNA is complementary with a sequence within exon 1 of CD3δ, CD3ε, or CD3γ.

[0018] In some examples, the complementary sequence of the guide RNA is within the CD3δ gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 53. In some examples, the complementary sequence of the guide RNA is within the CD3ε gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 52. In some examples, the complementary sequence of the guide RNA is within the CD3γ gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 54. In some examples, the complementary sequence of the guide RNA is within the B2M gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 55. In some examples, the complementary sequence of the guide RNA is within the CIITA gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 61. In some examples, the complementary sequence of the guide RNA is within the TAP1 gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 56. In

some examples, the complementary sequence of the guide RNA is within the TAP2 gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 57. In some examples, the complementary sequence of the guide RNA is within the TAPBP gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 58, SEQ ID NO: 59, or a combination thereof. In some examples, the complementary sequence of the guide RNA is within the NLRC5 gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 60. In some examples, the complementary sequence of the guide RNA is within the HLA-DM gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 62. In some examples, the complementary sequence of the guide RNA is within the RFX5 gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 63, SEQ ID NO: 64, or a combination thereof. In some examples, the complementary sequence of the guide RNA is within the RFXANK gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 65. In some examples, the complementary sequence of the guide RNA is within the RFXAP gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 66. In some examples, the complementary sequence of the guide RNA is within the Ii Chain gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 67, SEQ ID NO: 68, or any combination thereof.

[0019] In one example of the present disclosure, the modified immune cell exerts a reduced immune response in a subject when the modified immune cell is administered to the subject, as compared to the immune response exerted by an unmodified immune cell administered to the same subject.

[0020] In some examples, the modified immune cell exerts a reduced immune response in a subject when the modified immune cell is administered to the subject, as compared to the immune response exerted by an immune cell comprising an insertion and/or deletion capable of downregulating the gene expression of TRAC, TRBC, B2M, and CIITA. In some examples, the immune response is a graft-versus-host disease (GvHD) response. In some examples, the reduced GvHD response by the modified immune cell is compared to an equivalent immune cell without the deletion and/or insertion in one or more gene loci, or an immune cell comprising the deletion and/or insertion in TRAC, TRBC, B2M, and CIITA. In some examples, the reduced

GvHD response is elicited against an HLA-I mismatched cell or against an HLA-II mismatched cell.

[0021] In some examples, the GvHD response is reduced by about 10% or more, about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 70% or more, about 80% or more, about 90% or more, or about 95% or more. In some examples, the GvHD response is reduced by about 1-fold or more, about 2-fold or more, about 3-fold or more, about 4-fold or more, about 5-fold or more, about 6-fold or more, about 7-fold or more, about 8-fold or more, about 9-fold or more, about 10-fold or more, about 20-fold or more, about 30-fold or more, about 50-fold or more, about 100-fold or more, about 150-fold or more, or about 200-fold or more.

[0022] In one example of the present disclosure, the exogenous nucleic acid encodes a chimeric antigen receptor (CAR). In some examples, the CAR comprises an antigen binding domain, a hinge domain, a transmembrane domain, a costimulatory signaling domain, and an intracellular signaling domain. In some examples, the antigen-binding domain comprises a full length antibody or an antigen-binding fragment thereof, a Fab, a $F(ab)_2$, a monospecific Fab_2, a bispecific Fab_2, a trispecific Fab_2, a single-chain variable fragment (scFv), a diabody, a triabody, a minibody, a V-NAR, or a VhH.

[0023] In some examples of the modified immune cell, the transmembrane domain is selected from an artificial hydrophobic sequence, a transmembrane domain of a type I transmembrane protein, an alpha, beta, or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD2, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, OX40 (CD134), 4-1BB (CD137), ICOS (CD278), CD154, CD357 (GITR), Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, and a transmembrane domain derived from a killer immunoglobulin-like receptor (KIR).

[0024] In some examples of the modified immune cell, the costimulatory domain comprises one or more of a costimulatory domain of a protein selected from the group consisting of proteins in the TNFR superfamily, CD28, 4-1BB (CD137), OX40 (CD134), PD-1, CD7, LIGHT, CD83L, DAP10, DAP12, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40, ICOS (CD278), NKG2C, B7-H3 (CD276), and an intracellular domain derived from a killer immunoglobulin-like receptor (KIR), or a variant thereof.

[0025] In some examples of the modified immune cell, the intracellular signaling domain comprises an intracellular domain selected from the group consisting of cytoplasmic signaling domains of a human CD2, CD3 zeta chain (CD3ζ), FcγRIII, FcsRI, a cytoplasmic tail of an Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptor, TCR zeta, FcR gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d, or a variant thereof. In some examples, the intracellular signaling domain comprises a human CD3 zeta chain (CD3ζ).

[0026] In some examples of the modified immune cell, the antigen binding domain targets a tumor antigen associated with a hematologic malignancy, and/or associated with a solid tumor. In some examples, the antigen binding domain targets a tumor antigen selected from the group consisting of ROR1, mesothelin, c-Met, PSMA, PSCA, Folate receptor alpha, Folate receptor beta, EGFR, EGFRvIII, GPC2, GPC2, Mucin 1(MUC1), Tn antigen ((Tn Ag) or (GalNAca-Ser/Thr)), TnMUC1, GDNF family receptor alpha-4 (GFRa4), fibroblast activation protein (FAP), and Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2).

[0027] In some examples, the CAR comprises (a) a PSMA antigen binding domain, a CD2 costimulatory domain, and a CD3 zeta intracellular signaling domain; or (b) a mesothelin antigen binding domain, a 4-1BB costimulatory domain, and a CD3 zeta signaling domain; or (c) a TnMUC1 antigen binding domain, a CD2 costimulatory domain, and a CD3 zeta signaling domain.

[0028] In one example of the present disclosure, the modified immune cell further comprises a switch receptor. In some examples, the switch receptor comprises an extracellular domain of a signaling protein associated with a negative signal, a transmembrane domain, and an intracellular domain of a signaling protein associated with a positive signal.

[0029] In some examples, the modified immune cell further comprises a dominant negative receptor. In some examples, the dominant negative receptor comprises (a) a truncated variant of a wild-type protein associated with a negative signal; or (b) a variant of a wild-type protein associated with a negative signal comprising an extracellular domain, a transmembrane domain, and substantially lacking an intracellular signaling domain; or (c) an extracellular domain of a signaling protein associated with a negative signal, and a transmembrane domain. In some examples, the dominant negative receptor is PD1, VSIG3,

VISG8, or TGFβR dominant negative receptor.

[0030] In some examples, the protein associated with the negative signal is selected from the group consisting of CTLA4, PD-1, TGFβRII, BTLA, VSIG3, VSIG8, and TIM-3. In some examples, the protein associated with the positive signal is selected from the group consisting of CD28, 4-1BB, IL12Rβ1, IL12Rβ2, CD2, ICOS, and CD27.

[0031] In some examples, the switch receptor is selected from the group consisting of PD-1-CD28, PD-1^{A132L}-CD28, PD-1-CD27, PD-1^{A132L}-CD27, PD-1-4-1BB, PD-1^{A132L}-4-1BB, PD-1-ICOS, PD-1^{A132L}-ICOS, PD-1-IL12Rβ1, PD-1^{A132L}-IL12Rβ1, PD-1-IL12Rβ2, PD-1^{A132L}-IL12Rβ2, VSIG3-CD28, VSIG8-CD28, VSIG3-CD27, VSIG8-CD27, VSIG3-4-1BB, VSIG8-4-1BB, VSIG3-ICOS, VSIG8-ICOS, VSIG3-IL12Rβ1, VSIG8-IL12Rβ1, VSIG3-IL12Rβ2, VSIG8-IL12Rβ2, TGFβRII-CD27, TGFβRII-CD28, TGFβRII-4-1BB, TGFβRII-ICOS, TGFβRII-IL12Rβ1, and TGFβRII-IL12Rβ2.

[0032] In some examples of the modified immune cell, the transmembrane domain of the switch receptor is selected from a transmembrane domain of a protein selected from the group consisting of CTLA4, PD-1, VSIG3, VSIG8, TGFβRII, BTLA, TIM-3, CD28, 4-1BB, IL12Rβ1, IL12Rβ2, CD2, ICOS, and CD27. In some examples, the transmembrane domain of the switch receptor is selected from the transmembrane of the protein associated with a negative signal or the transmembrane domain of the protein associated with the negative signal.

[0033] In one example, the present disclosure provides an isolated modified T cell, comprising at least one functionally impaired polypeptide selected from the group consisting of CD3δ, CD3ε, CD3γ, B2M, C2TA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, and invariant chain (Ii Chain). In some examples, the modified T cell comprising the functionally impaired polypeptide exhibits at least one of (i) reduced T cell receptor expression as compared to an unmodified T cell; (ii) reduced expression of the impaired polypeptide; (iii) complete absence of the T cell receptor complex surface expression; and/or (iv) reduced or insufficient T cell receptor cross-linking. In one aspect, the present disclosure refers to an isolated modified T cell, comprising at least one functionally impaired polypeptide selected from the group consisting of CD3δ, CD3ε, CD3γ, B2M, C2TA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, and invariant chain (Ii Chain); wherein the modified T cell comprising the functionally impaired polypeptide exhibits at least one of: (i) reduced T cell receptor expression as compared to an unmodified T cell; (ii) reduced expression of the impaired polypeptide; (iii) complete absence of the T cell receptor complex surface expression; and (iv) reduced or insufficient T cell receptor cross-linking. In some examples, the T cell exerts a reduced immune response in a subject when the modified T cell is administered to the subject, as compared to the immune response exerted by an unmodified T cell administered to the same subject.

[0034] In some examples, the T cell comprises two or more functionally impaired

polypeptides, and wherein the second impaired polypeptide is T-cell receptor α chain (TRAC) and/or T-cell receptor β chain (TRBC).

[0035] In some examples, the modified T cell comprises: (a) three or more functionally impaired polypeptides selected from TRAC, CD3δ, CD3ε, CD3γ, B2M, C2TA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, or Ii chain; or (b) two functionally impaired polypeptides selected from CD3α, CD3δ, CD3ε, CD3γ, B2M, C2TA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, or Ii chain; or (c) three functionally impaired polypeptides selected from CD3α, CD3δ, CD3ε, CD3γ, B2M, C2TA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, or Ii chain; or (d) a functionally impaired polypeptide selected from the group consisting of CD3δ, CD3ε, and CD3γ, and at least one functionally impaired polypeptide selected from TRAC, TRBC, B2M, C2TA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, or Ii Chain; or (e) a functionally impaired polypeptide selected from the group consisting of CD3δ, CD3ε, and CD3γ, and a functionally impaired polypeptide selected from TRAC, TRBC, B2M, C2TA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, or Ii Chain.

[0036] In some examples, the modified T cell comprises: (a) functionally impaired CD3δ and at least one functionally impaired polypeptide selected from TRAC, TRBC, B2M, C2TA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, or Ii chain; (b) functionally impaired CD3ε and at least one functionally impaired polypeptide selected from TRAC, TRBC, B2M, C2TA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, or Ii chain; or (c) functionally impaired CD3γ and at least one functionally impaired polypeptide selected from TRAC, B2M, C2TA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, or Ii chain.

[0037] In some examples, the modified T cell comprises two or more functionally impaired polypeptides selected from TRAC, TRBC, B2M, C2TA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, or Ii chain. In some examples, the modified T cell has a reduced expression of TRAC, TRBC, CD3δ, CD3ε, CD3γ, B2M, C2TA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, Ii chain, or any combination thereof. In some examples, the modified T cell does not express CD3δ, CD3ε, CD3γ, TRAC, B2M, C2TA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, Ii chain, or

any combination thereof.

[0038] In some examples, the modified T cell further comprises a functionally impaired polypeptide selected from TRAC, TRBC, B2M, and C2TA. In some examples, the modified T cell has a reduced expression of TRAC, TRBC, B2M, or C2TA or does not express TRAC, TRBC, B2M, or C2TA. In some examples, the modification of CD3δ, CD3ε, and/or CD3γ leads to an impaired TCR/CD3 complex function. In some examples, at least one of CD3δ, CD3ε, or CD3γ is modified by targeting one or more exons of CD3δ, CD3ε, or CD3γ, optionally exon 1 of CD3δ, CD3ε, or CD3γ.

[0039] One example of the present disclosure provides a method for generating a modified immune cell comprising: (a) introducing into the immune cell one or more nucleic acids capable of downregulating gene expression of one or more endogenous immune genes encoding an endogenous immune protein selected from the group consisting of CD3δ, CD3ε, CD3γ, B2M, CIITA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, and invariant chain (Ii Chain); (b) introducing into the immune cell an exogenous nucleic acid encoding a chimeric antigen receptor (CAR), an engineered T cell receptor (TCR), a Killer cell immunoglobulin-like receptor (KIR), an antigen-binding polypeptide, a cell surface receptor ligand, or a tumor antigen; and (c) expanding the modified immune cell to generate a population of T cells. In some examples, the method for generating a modified immune cell further comprising introducing into the immune cell an exogenous nucleic acid encoding a dominant negative receptor, a switch receptor, or a combination thereof. In one aspect, the present disclosure refers to a method for generating a modified immune cell comprising: (a) introducing into the immune cell one or more nucleic acids capable of downregulating gene expression of one or more endogenous immune genes encoding an endogenous immune protein selected from the group consisting of CD3δ, CD3ε, CD3γ, B2M, CIITA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, and invariant chain (Ii Chain); (b) introducing into the immune cell an exogenous nucleic acid encoding a chimeric antigen receptor (CAR), an engineered T cell receptor (TCR), a Killer cell immunoglobulin-like receptor (KIR), an antigen-binding polypeptide, a cell surface receptor ligand, or a tumor antigen; and (c) expanding the modified immune cell to generate a population of T cells; and optionally (d) further comprising introducing into the immune cell an exogenous nucleic acid encoding a dominant negative receptor, a switch receptor, or a combination thereof.

[0040] In some examples, the one or more nucleic acids introduced into the modified immune cell are capable of downregulating the gene expression of: (a) a T cell receptor subunit selected from CD3δ, CD3ε, or CD3γ; and/or (b) a HLA class I molecule selected from B2M, TAP1, TAP2, TAPBP, or NLRC5; and/or (c) a HLA class II molecule selected from HLA-DM, RFX5, RFXANK, RFXAP, or invariant chain (Ii Chain).

[0041] In some examples, the one or more nucleic acids introduced into the modified immune cell are capable of downregulating the gene expression of CD3δ, and the gene expression of a HLA molecule selected from the group consisting of B2M, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, invariant chain (Ii Chain), and a combination thereof. In some examples, the one or more nucleic acids introduced into the

modified immune cell are capable of downregulating the gene expression of CD3ε, and a HLA molecule selected from the group consisting of B2M, TAP1, TAP2, TAPBP, NLRC5, CIITA, HLA-DM, RFX5, RFXANK, RFXAP, invariant chain (Ii Chain), and a combination thereof. In some examples, the one or more nucleic acids introduced into the modified immune cell are capable of downregulating the gene expression of CD3γ, and a HLA molecule selected from the group consisting of B2M, TAP1, TAP2, TAPBP, NLRC5, CIITA, HLA-DM, RFX5, RFXANK, RFXAP, invariant chain (Ii Chain), and a combination thereof.

[0042] In some examples, the one or more nucleic acids introduced into the modified immune cell are capable of downregulating the gene expression of (a) CD3ε, B2M, and CIITA; (b) CD3ε, B2M, and RFX5; (c) CD3ε, B2M, and RFXAP; (d) CD3ε, B2M, and RFXANK; (e) CD3ε, B2M, and HLA-DM; (f) CD3ε, B2M, and Ii chain; (g) CD3ε, TAP1, and CIITA; (h) CD3ε, TAP1, and RFX5; (i) CD3ε, TAP1, and RFXAP; (j) CD3ε, TAP1, and RFXANK; (k) CD3ε, TAP1, and HLA-DM; (l) CD3ε, TAP1, and Ii chain; (m) CD3ε, TAP2, and CIITA; (n) CD3ε, TAP2, and RFX5; (o) CD3ε, TAP2, and RFXAP; (p) CD3ε, TAP2, and RFXANK; (q) CD3ε, TAP2, and HLA-DM; (r) CD3ε, TAP2, and Ii chain; (s) CD3ε, NLRC5, and CIITA; (t) CD3ε, NLRC5, and RFX5; (u) CD3ε, NLRC5, and RFXAP; (v) CD3ε, NLRC5, and RFXANK; (w) CD3ε, NLRC5, and HLA-DM; (x) CD3ε, NLRC5, and Ii chain; (y) CD3ε, TAPBP, and CIITA; (z) CD3ε, TAPBP, and RFX5; (aa) CD3ε, TAPBP, and RFXAP; (bb) CD3ε, TAPBP, and RFXANK; (cc) CD3ε, TAPBP, and HLA-DM; or (dd) CD3ε, TAPBP, and Ii chain.

[0043] In some examples, the one or more nucleic acids introduced into the modified immune cell are capable of downregulating the gene expression of: (a) CD3δ, B2M, and CIITA; (b) CD3δ, B2M, and RFX5; (c) CD3δ, B2M, and RFXAP; (d) CD3δ, B2M, and RFXANK; (e) CD3δ, B2M, and HLA-DM; (f) CD3δ, B2M, and Ii chain; (g) CD3δ, TAP1, and CIITA; (h) CD3δ, TAP1, and RFX5; (i) CD3δ, TAP1, and RFXAP; (j) CD3δ, TAP1, and RFXANK; (k) CD3δ, TAP1, and HLA-DM; (l) CD3δ, TAP1, and Ii chain; (m) CD3δ, TAP2, and CIITA; (n) CD3δ, TAP2, and RFX5; (o) CD3δ, TAP2, and RFXAP; (p) CD3δ, TAP2, and RFXANK; (q) CD3δ, TAP2, and HLA-DM; (r) CD3δ, TAP2, and Ii chain; (s) CD3δ, NLRC5, and CIITA; (t) CD3δ, NLRC5, and RFX5; (u) CD3δ, NLRC5, and RFXAP; (v) CD3δ, NLRC5, and RFXANK; (w) CD3δ, NLRC5, and HLA-DM; (x) CD3δ, NLRC5, and Ii chain; (y) CD3δ, TAPBP, and CIITA; (z) CD3δ, TAPBP, and RFX5; (aa) CD3δ, TAPBP, and RFXAP; (bb) CD3δ, TAPBP,

and RFXANK; (cc) CD3δ, TAPBP, and HLA-DM; or (dd) CD3δ, TAPBP, and Ii chain.

[0044] In some examples, the one or more nucleic acids introduced into the modified immune cell are capable of downregulating the gene expression of: (a) CD3γ, B2M, and CIITA; (b) CD3γ, B2M, and RFX5; (c) CD3γ, B2M, and RFXAP; (d) CD3γ, B2M, and RFXANK; (e) CD3γ, B2M, and HLA-DM; (f) CD3γ, B2M, and Ii chain; (g) CD3γ, TAP1, and CIITA; (h) CD3γ, TAP1, and RFX5; (i) CD3γ, TAP1, and RFXAP; (j) CD3γ, TAP1, and RFXANK; (k) CD3γ, TAP1, and HLA-DM; (l) CD3γ, TAP1, and Ii chain; (m) CD3γ, TAP2, and CIITA; (n) CD3γ, TAP2, and RFX5; (o) CD3γ, TAP2, and RFXAP; (p) CD3γ, TAP2, and RFXANK; (q) CD3γ, TAP2, and HLA-DM; (r) CD3γ, TAP2, and Ii chain; (s) CD3γ, NLRC5, and CIITA; (t) CD3γ, NLRC5, and RFX5; (u) CD3γ, NLRC5, and RFXAP; (v) CD3γ, NLRC5, and RFXANK; (w) CD3γ, NLRC5, and HLA-DM; (x) CD3γ, NLRC5, and Ii chain; (y) CD3γ, TAPBP, and CIITA; (z) CD3γ, TAPBP, and RFX5; (aa) CD3γ, TAPBP, and RFXAP; (bb) CD3γ, TAPBP, and RFXANK; (cc) CD3γ, TAPBP, and HLA-DM; or (dd) CD3γ, TAPBP, and Ii chain.

[0045] In some examples, the immune cell to be modified is selected from the group consisting of a T cell, a natural killer cell (NK cell), a natural killer T cell, a lymphoid progenitor cell, a hematopoietic stem cell, a stem cell, a macrophage, and a dendritic cell. In some examples, the immune cell to be modified the immune cell is a CD4+ T cell or a CD8+ T cell. In some examples, the immune cell to be modified is an allogeneic T cell or autologous T cell.

[0046] In some examples, the nucleic acids are introduced into the immune cell by viral transduction. In some examples, the viral transduction comprises contacting the immune cell with a viral vector comprising the one or more nucleic acids. In some examples, the viral vector is selected from the group consisting of a retroviral vector, sendai viral vectors, adenoviral vectors, adeno-associated virus vectors, and lentiviral vectors.

[0047] In some examples, each of the one or more nucleic acids that are capable of downregulating expression of one or more endogenous immune genes comprises a gene editing system selected from the group consisting of: (a) a CRISPR-associated (Cas) (CRISPR-CAs) endonuclease system and a guide RNA; (b) a TALEN gene editing system, a zinc finger nuclease (ZFN) gene editing system, a meganuclease gene editing system, or a mega-TALEN gene editing system; and (c) a gene silencing system selected from antisense RNA, antigomer RNA, RNAi,

siRNA, or shRNA.

[0048] In some examples, the Cas endonuclease comprises Cas3, Cas4, Cas8a, Cas8b, Cas9, Cas10, Cas10d, Cas12a, Cas12b, Cas12d, Cas12e, Cas12f, Cas12g, Cas12h, Cas12i, Cas13, Cas14, CasX, Cse1, Csy1, Csn2, Cpf1, C2c1, Csm2, Cmr5, Fok1, *S. pyogenes* Cas9, *Staphylococcus aureus* Cas9, MAD7 nuclease (a type V CRISPR nuclease), or any combination thereof. In some examples, the CRISPR-Cas system comprises an pAd5/F35-CRISPR vector.

[0049] In some examples, the guide RNA of the CRISPR-Cas system comprises a guide sequence that is complementary with a sequence within the one or more gene loci each encoding the immune protein selected from the group consisting of CD3δ, CD3ε, CD3γ, B2M, CIITA, TAP1, TAP2, TAPBP, NLRC5, HLA-DM, RFX5, RFXANK, RFXAP, and invariant chain (Ii Chain).

[0050] In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within one or more exons of CD3δ, CD3ε, or CD3γ. In some examples, the guide RNA is complementary with a sequence within exon 1 of CD3δ, CD3ε, or CD3γ.

[0051] In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the CD3δ gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 53. In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the CD3ε gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 52. In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the CD3γ gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 54. In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the B2M gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 55. In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the CIITA gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 61. In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the TAP1

gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 56. In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the TAP2 gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 57. In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the TAPBP gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 58, SEQ ID NO: 59, or any combination thereof. In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the NLRC5 gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 60. In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the HLA-DM gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 62. In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the RFX5 gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 63, SEQ ID NO: 64, or any combination thereof. In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the RFXANK gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 65. In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the RFXAP gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 66. In some examples, the guide RNA introduced into the modified immune cell is complementary with a sequence within the Ii Chain gene locus and the guide RNA comprises a nucleic acid sequence encoded by SEQ ID NO: 67, SEQ ID NO: 68, or any combination thereof.

[0052] In some examples, the modified immune cell generated by the disclosed method exerts a reduced immune response in a subject when the modified immune cell is administered to a subject, as compared to the immune response exerted by an unmodified immune cell administered to the same subject.

[0053] In some example, the modified immune cell the modified immune cell generated by the disclosed method exerts a reduced immune response in a subject when the immune cell is administered to the subject, as compared to the immune response exerted by an immune cell comprising one or more nucleic acids capable of downregulating the gene expression of TRAC, TRBC, B2M, and CIITA. In some examples, the reduced GvHD response by the modified

immune cell generated by the disclosed method is compared to an equivalent immune cell without the deletion and/or insertion in one or more gene loci, or an immune cell comprising the deletion and/or insertion in TRAC, TRBC, B2M, and CIITA.

[0054] In some examples, the immune response is a graft-versus-host disease (GvHD) response. In some examples, the reduced GvHD response is elicited against an HLA-I mismatched cell or against an HLA-II mismatched cell. In some examples, the GvHD response elicited by a modified immune cell generated by the disclosed method is reduced by about 10% or more, about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 70% or more, about 80% or more, about 90% or more, or about 95% or more. In some examples, the GvHD response elicited by a modified immune cell generated by the disclosed method is reduced by about 1-fold or more, about 2-fold or more, about 3-fold or more, about 4-fold or more, about 5-fold or more, about 6-fold or more, about 7-fold or more, about 8-fold or more, about 9-fold or more, about 10-fold or more, about 20-fold or more, about 30-fold or more, about 50-fold or more, about 100-fold or more, about 150-fold or more, or about 200-fold or more.

[0055] In some examples, the exogenous nucleic acid introduced into the immune cell encodes a chimeric antigen receptor (CAR). In some examples, the CAR comprises an antigen binding domain, a hinge domain, a transmembrane domain, a costimulatory signaling domain, and an intracellular signaling domain.

[0056] In some examples, the antigen binding domain targets a tumor antigen associated with a hematologic malignancy; and/or associated with a solid tumor. In some examples, the antigen binding domain targets a tumor antigen selected from the group consisting of ROR1, mesothelin, c-Met, PSMA, PSCA, Folate receptor alpha, Folate receptor beta, EGFR, EGFRvIII, GPC2, GPC2, Mucin 1(MUC1), Tn antigen ((Tn Ag) or (GalNAca-Ser/Thr)), TnMUC1, GDNF family receptor alpha-4 (GFRa4), fibroblast activation protein (FAP), and Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2).

[0057] In some examples, the CAR introduced into the modified immune cell comprises: (a) a PSMA antigen binding domain (e.g. SEQ ID NOs: 73 or 74), a CD2 costimulatory domain, and a CD3 zeta intracellular signaling domain; or (b) a mesothelin antigen binding domain (e.g., SEQ ID NO: 75), a 4-1BB costimulatory domain, and a CD3 zeta signaling domain; or (c) a

TnMUC1 antigen binding domain, a CD2 costimulatory domain, and a CD3 zeta signaling domain. In some examples, the TnMUC1 CAR comprises an amino acid sequence set forth in SEQ ID NO: 70, and the mesothelin CAR comprises an amino acid sequence set forth in SEQ ID NO: 71 or SEQ ID NO: 72. In some examples, the TnMUC1 CAR is encoded by a nucleic acid sequence set forth in SEQ ID NO: 69.

[0058] In one example of the disclosure, the switch receptor introduced in the modified immune cell comprises: (a) an extracellular domain of a signaling protein associated with a negative signal selected from the group consisting of CTLA4, PD-1, VISG3, VSIG8, TGFβRII, BTLA, and TIM-3, (b) a transmembrane domain, and (c) an intracellular domain of a signaling protein associated with a positive signal selected from the group consisting of CD28, 4-1BB, IL12Rβ1, IL12Rβ2, CD2, ICOS, and CD27.

[0059] In some examples, the switch receptor is selected from the group consisting of PD-1-CD28, PD-1^{A132L}-CD28, PD-1-CD27, PD-1^{A132L}-CD27, PD-1-4-1BB, PD-1^{A132L}-4-1BB, PD-1-ICOS, PD-1^{A132L}-ICOS, PD-1-IL12Rβ1, PD-1^{A132L}-IL12Rβ1, PD-1-IL12Rβ2, PD-1^{A132L}-IL12Rβ2, VSIG3-CD28, VSIG8-CD28, VSIG3-CD27, VSIG8-CD27, VSIG3-4-1BB, VSIG8-4-1BB, VSIG3-ICOS, VSIG8-ICOS, VSIG3-IL12Rβ1, VSIG8-IL12Rβ1, VSIG3-IL12Rβ2, VSIG8-IL12Rβ2, TGFβRII-CD27, TGFβRII-CD28, TGFβRII-4-1BB, TGFβRII-ICOS, TGFβRII-IL12Rβ1, and TGFβRII-IL12Rβ2.

[0060] In some examples, the dominant negative receptor introduced into the modified immune cell comprises: (a) a truncated variant of a wild-type protein associated with a negative signal, or (b) a variant of a wild-type protein associated with a negative signal comprising an extracellular domain, a transmembrane domain, and substantially lacking an intracellular signaling domain; or (c) an extracellular domain of a signaling protein associated with a negative signal, and a transmembrane domain. In some examples, the dominant negative receptor is PD1, VSIG3, VSIG8, or TGFβR dominant negative receptor.

[0061] In some examples, the transmembrane domain of the switch receptor is selected from a transmembrane domain of a protein selected from the group consisting of CTLA4, PD-1, BTLA, TGFβRII, BTLA, TIM-3, CD28, 4-1BB, IL12Rβ1, IL12Rβ2, CD2, ICOS, and CD27. In some examples, the transmembrane domain of the switch receptor is selected from the transmembrane of the protein associated with a negative signal or the transmembrane domain of

the protein associated with the negative signal.

[0062] One example of the present disclosure provides expanding the modified immune cell. In some examples, expanding the modified immune cell comprises culturing the T cell with a factor selected from the group consisting of flt3-L, IL-1, IL-3, IL-2, IL-7, IL-15, IL-18, IL-21, TGFbeta, IL-10, and c-kit ligand. One example of the present disclosure provides further introducing a polypeptide and/or a nucleic acid encoding Klf4, Oct3/4 and Sox2 in the immune cell to induce pluripotency of the immune cell.

[0063] In some examples, the immune cell is obtained from a blood sample, a whole blood sample, a peripheral blood mononuclear cell (PBMC) sample, or an apheresis sample. In some examples, the apheresis sample is a cryopreserved sample. In some examples, the apheresis sample is a fresh sample. In some examples, the immune cell is obtained from a human subject.

[0064] One example of the present disclosure provides a population of modified immune cells obtained by the method of any one of the preceding examples. In one aspect, the present disclosure refers to a population of modified immune cells obtained from the method as disclosed herein. In some examples, the composition comprises the modified immune cell of any one of the preceding examples. In some examples, the composition comprises a population of modified immune cells generated by the methods disclosed in any one of the preceding examples and pharmaceutically acceptable carrier or excipient. In one aspect, the present disclosure refers to a composition comprising the modified immune cell or modified T cell as disclosed herein, or the population of modified immune cells as disclosed herein, and a pharmaceutically acceptable carrier or excipient.

[0065] One example of the present disclosure provide a method of treating a disease or condition associated with enhanced immunity in a subject comprising administering an effective amount of the composition disclosed in any one of the preceding examples to a subject in need thereof. In one aspect, the present disclosure refers to use of the composition as disclosed herein in the manufacture of a medicament for treating a disease or condition associated with enhanced immunity in a subject in need thereof. In some examples, the condition is a cancer. In some examples, the cancer is selected from the group consisting of breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer, and any combination thereof. In some examples, the cancer is a solid tumor, or a hematologic malignancy. In some examples, a method of treating a cancer comprises administering to a subject the modified immune cells of in any one of the preceding examples, the population of modified T cells of any one of the preceding examples, or the composition of any one of the preceding examples. In one aspect, the present disclosure refers to use of the modified immune cells or modified T cells as disclosed herein, the population of modified immune cells as disclosed herein, or the composition as disclosed herein in the manufacture of a medicament for treating a cancer.

[0066] One example of the present disclosure provides a method for stimulating a T cell-

mediated immune response to a target cell or tissue in a subject comprising administering to a subject an effective amount of a pharmaceutical composition comprising the modified immune cell of any one of the preceding examples, the population of modified immune cells of any one of the preceding examples, or the composition of any one of the preceding examples. In one aspect, the present disclosure refers to use of a pharmaceutical composition comprising the modified immune cell or modified T cell as disclosed herein, the population of modified immune cells as disclosed herein, or the composition as disclosed herein in the manufacture of a medicament for stimulating a T cell-mediated immune response to a target cell or tissue in a subject.

[0067] One example of the present disclosure provide a kit comprising the modified immune cells of any one of the preceding examples, the population of modified T cells of any one of the preceding examples, or the composition of any one of the preceding examples, optionally comprising an instruction for use. In one aspect, the present disclosure refers to a kit comprising the modified immune cells as disclosed herein, the population of modified T cells as disclosed herein, or the composition as disclosed herein, optionally comprising an instruction for use.

[0068] Both the foregoing summary and the following description of the drawings and detailed description are exemplary and explanatory. They are intended to provide further details of the disclosure, but are not to be construed as limiting. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following detailed description of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0069] **FIG. 1** shows a schematic representation of the human T-cell receptor (TCR) –CD3 complex, comprising the variable TCR-alpha chain (TCR-α, TRAC), and TCR-beta chain (TCR-β, TRBC) coupled to three dimeric modules CD3δ/CD3ε, CD3γ/CD3ε, and CD3ζ/CD3ζ. The CD3δ/CD3ε, and CD3γ/CD3ε modules are the subject of the present disclosure.

[0070] **FIGs. 2A-2C** show bar graphs illustrating the TCR-α and TCR-β chains disruption efficiency on human T cells, as measured by flow cytometry following the targeted disruption of CD3δ (**FIG. 2A**), CD3ε (**FIG. 2B**), and CD3γ (**FIG. 2C**) genes using a CRISPR/Cas system.

[0071] **FIG. 3** shows a graph illustrating the expansion of allogeneic CART-cells generated using the strategy of FIG. 1, and illustrates the number of population doublings over a ten-day period. The tested allogeneic CART cells are engineered T cells comprising TRAC knockout (TRAC KO), CD3δ knockout (D1 KO), CD3γ knockout (G4 KO), and CD3ε knockout (E4 KO).

[0072] **FIGs. 4A and 4B** show flow cytometry results comparing CRISPR-mediated downregulation of TCR-α chain (TRAC) knockout, CD3δ knockout (D1 KO), CD3γ knockout (G4 KO), and CD3ε knockout (E4 KO). **FIG. 4A** shows that CD3ε knockout (E4 KO) is a better target for T cell receptor knockout, as measured by surface expression of the TCR-α/β chain.