

ENHANCED TUMOR REACTIVITY OF T CELLS LACKING SIT1, LAX1, OR TRAT1

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 63/625,354, filed January 26, 2024, which is incorporated by reference in its entirety herein.

STATEMENT REGARDING
FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with Government support under project number ZIAHD001803-28 by the National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development. The Government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED
ELECTRONICALLY

[0003] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 74,966 Byte Extensible Markup Language (XML) file named "772005.xml," dated January 23, 2025.

BACKGROUND OF THE INVENTION

[0004] Immunotherapy can be an effective treatment for a variety of conditions in some patients. However, obstacles to the overall success of immunotherapy still exist. For example, reactivity against a target antigen can be attenuated. Despite considerable research in the field of immunotherapy, there still exists a need for improved methods and products for immunotherapy.

BRIEF SUMMARY OF THE INVENTION

[0005] An aspect of the invention provides a T cell expressing an antigen-specific receptor, wherein the T cell has been modified to reduce one or both of expression and activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1*.

[0006] Another aspect of the invention provides a nucleic acid comprising a nucleotide sequence encoding one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1*.

[0007] Aspects of the invention further provide related recombinant expression vectors, mutant proteins, host cells, populations of cells, and pharmaceutical compositions relating to the T cells and nucleic acids of the invention.

[0008] Another aspect of the invention provides a method of treating a condition in a mammal comprising administering to the mammal any of the inventive T cells, populations of cells, nucleic acids, mutant proteins, recombinant expression vectors, host cells, or pharmaceutical compositions in an amount effective to treat the condition in the mammal, wherein the condition is cancer, an infectious disease, or an autoimmune disease.

[0009] Another aspect of the invention provides a method of enhancing an antigen-specific immune response in a mammal, the method comprising administering to the mammal any of the inventive T cells, populations of cells, nucleic acids, mutant proteins, recombinant expression vectors, host cells, or pharmaceutical compositions in an amount effective to enhance the antigen-specific immune response in the mammal.

[0010] Additional aspects of the invention provide methods of making the inventive modified T cells.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0011] Figures 1A-1D are graphs showing the results of an xCELLigence™ (Agilent) assay showing growth (cell index) of N4 or V4-peptide expressing B16F10 murine melanoma cells (1A and 1B) or MC38 murine colorectal carcinoma cells (1C and 1D) at the indicated number of hours after addition of CD8+ effector T cells from control *OT1-tg; dLckCre* mice or from *OT1-tg; dLckCre; Sit1* fl/fl mice. 10,000 B16F10 or MC38 cells were plated in each well. Effector T cell: B16F10 melanoma cell or Effector T cell: MC38 colon carcinoma cell ratio used: 10, 5, 2.5, 1.25, 0.63, 0.31 and 0.16. TRITON X-100 detergent solution (Thermo Fisher Scientific Inc., Waltham, MA) served as a positive control for cell lysis. No effector CD8+ T cells were added to the untreated samples. For simplicity, E:T 1.25 and 2.5 are shown for B16F10-N4 (1A), E:T 0.63 and 2.5 are shown for MC38-N4 (1B), E:T 2.5 and 10 for B16F10-V4 melanoma cells (1C) and E:T 1.25 and 10 for MC38-V4 colorectal cells (1D). Data are represented as mean $\pm$ SEM (n=3). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (*p<0.05; **p<0.005; ****p<0.0001).

[0012] Figures 2A-2D are graphs showing the results of an xCELLigence™ assay showing growth (cell index) of N4 or V4-peptide expressing B16F10 melanoma cells (2A and 2C) and MC38 colorectal carcinoma cells (2B and 2D) at the indicated number of hours after addition of CD8+ effector T cells from control *OT1-tg;dLCK-Cre* mice and from *OT1-tg; dLCK-Cre;Lax1 fl/fl* mice. 10,000 B16F10 or MC38 cells were plated in each well. Effector T cell: melanoma cell or Effector T cell: MC38 cell ratio used: 10, 5, 2.5, 1.25, 0.63, 0.31 and 0.16. TRITON X-100 detergent solution served as a positive control for cell lysis. No effector CD8+ T cells were added to the untreated samples. For simplicity, E:T 0.63 and 1.25 are shown for B16F10-N4 (2A), E:T 1.25 and 2.5 are shown for MC38-N4 (2B), E:T 5 and 10 for B16F10-V4 melanoma cells (2C) and E:T 2.5 and 5 for MC38-V4 colorectal cells (2D). Data are represented as mean $\pm$ SEM (n=3). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (**p<0.005; ***p<0.0005; ****p<0.0001).

[0013] Figures 3A-3D are graphs showing the results of an xCELLigence™ assay showing growth (cell index) of N4 or V4-peptide expressing B16F10 melanoma cells (3A and 3C) and MC38 colorectal carcinoma cells (3B and 3D) at the indicated number of hours after addition of CD8+ effector T cells from control *OT1-tg;dLCK-Cre* mice and from *OT1-tg; dLCK-Cre;Trat1 fl/fl* mice. 10,000 B16F10 or MC38 cells were plated in each well. Effector T cell: melanoma cell or Effector T cell: MC38 cell ratio used: 10, 5, 2.5, 1.25, 0.63, 0.31 and 0.16. TRITON X-100 detergent solution served as a positive control for cell lysis. No effector CD8+ T cells were added to the untreated samples. For simplicity E:T 0.31 and 1.25 are shown for B16F10-N4 (3A), E:T 0.63 and 1.25 are shown for both MC38-N4 and V4 cells (3B and 3D) and E:T 5 and 10 for B16F10-V4 melanoma cells (3C). Data are represented as mean $\pm$ SEM (n=3). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (**p<0.005; ***p<0.0005; ****p<0.0001).

[0014] Figures 4A-4B show dot plots indicating the expression of CD8 (y axis) and LNGFR (x axis) measured by flow cytometry. CD8+ OT1 T cell antigen receptor (TCR)-tg spleen cells were untransduced (4A) or transduced with empty control retroviral vector (4A), or a retroviral vector encoding SIT1 WT (4A), SIT1 where the cytoplasmic domain has been deleted (c-terminal truncated only) (4B), SIT1 c-terminal truncation+ a sequence encoding an ITAM signaling motif from the TCR CD3zeta chain (Sit1 truncated + CD3 ITAM) (4B) or SIT1 c-terminal truncation + an activating motif sequence from the CD28 coreceptor (Sit1 truncated + CD28) (4B) constructs. Five days after retroviral transduction, retroviral

transduction efficiency was measured by expression of the LNGFR reporter marker by flow cytometry. Cells were activated by CD3+CD28 antibody stimulation prior to transduction.

[0015] Figures 4C-4J are graphs showing the results of an xCELLigence™ assay showing growth (cell index) of N4 and V4-peptide expressing B16F10 melanoma cells at the indicated number of hours that were untreated or after addition of CD8+ effector T cells from OT1 TCR-tg mice that were transduced with control empty vector (OTI vector), WT SIT1 (OTI_SIT1 WT) (4C, 4G), SIT1c-terminal truncated (OTI_SIT1 truncated) (4D, 4H), SIT1c-terminal truncation+CD3z ITAM (OTI_SIT1 truncated_CD3ITAM) (4E, 4I) or SIT1 c-terminal truncation+ CD28 active motif (OTI_SIT1 truncated_CD28) (4F, 4J). 10,000 B16F10-N4 or B16F10-V4 melanoma cells were plated in each well. Effector T cell: melanoma cell ratio used: 10, 5, 2.5, 1.25, 0.63, 0.31 and 0.16. TRITON X-100 detergent solution served as a positive control for cell lysis. No effector cells were added for untreated cells. For simplicity, E:T 1.25 is shown for both B16F10-N4 (4C-4F) and -V4 (4G-4J) melanoma cells. Data are represented as mean $\pm$ SEM (n=3). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (*p<0.05; **p<0.005; ***p<0.0005; ****p<0.0001).

[0016] Figures 5A-5B show dot plots indicating the expression of CD8 and LNGFR measured by flow cytometry. CD8+ OT1-tg spleen T cells were untransduced (5A) or transduced with vector control (Vector only) (5A), LAX1 WT (5B), or LAX1 c-terminal truncated (LAX1 Truncated only) (5B) constructs. After 5 days of transduction, efficiency was measured by expression of LNGFR reporter marker by flow cytometry. Cells were activated by CD3+CD28 antibodies prior to transduction.

[0017] Figures 5C-5D are graphs showing the results of an xCELLigence™ assay showing growth (cell index) of N4 or V4-peptide expressing B16F10 melanoma cells at the indicated number of hours that were untreated or after addition of CD8+ effector T cells from OT1 TCR-tg mice that were transduced with control empty vector (OTI vector), WT LAX1 (OTI_LAX1 WT), or LAX1 c-terminal truncated (OTI_LAX1 Truncated) constructs. 10,000 B16F10 cells were plated in each well. Effector T cell: melanoma cell ratio used: 10, 5, 2.5, 1.25, 0.63, 0.31 and 0.16. TRITON X-100 detergent solution served as a positive control for cell lysis. No effector cells were added for untreated cells. For simplicity E:T 1.25 is shown for both B16F10-N4 (5C) and -V4 (5D) melanoma cells. Data are represented as mean $\pm$ SEM (n=3). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (*p<0.05; **p<0.005; ***p<0.0005; ****p<0.0001).

[0018] Figures 6A-6B show dot plots indicating the expression of CD8 and LNGFR measured by flow cytometry. CD8⁺ OT1-tg spleen cells were untransduced (6A) or transduced with vector control (Vector only) (6A), WT TRAT (TRAT1 WT) (6A), TRAT1 c-terminal truncated only (6B), TRAT1 c-terminal truncation + CD3zeta ITAM (6B) or SIT1 c-terminal truncation + CD28 active motif (6B) constructs. After 5 days of transduction, efficiency was measured by expression of LNGFR reporter marker by flow cytometry. Cells were activated by CD3+CD28 antibodies prior to transduction.

[0019] Figures 6C-6H are graphs showing the results of an xCELLigence™ assay showing growth of N4 or V4-peptide expressing B16F10 melanoma cells at the indicated number of hours that were untreated or after addition of CD8⁺ effector T cells from OT1 TCR-tg mice transduced with control retroviral vector (OTI vector), WT TRAT1 (OTI_TRAT1 WT) (6C, 6F), TRAT1 c-terminal truncated only (OTI_TRAT1 truncated) (6D, 6E, 6G, 6H), TRAT1 c-terminal truncation+CD3zeta ITAM (OTI_TRAT1 truncated_CD3 ITAM) (6D, 6G), TRAT1 c-terminal truncation+ CD28 active motif (OTI_TRAT1 truncated_CD28) (6E, 6H) retroviral vectors. 10,000 B16F10 cells were plated in each well. Effector T cell: melanoma cell ratio used: 10, 5, 2.5, 1.25, 0.63, 0.31 and 0.16. TRITON X-100 detergent solution served as a positive control for cell lysis. No effector cells were added for untreated cells. For simplicity, E:T 1.25 is shown for both B16F10-N4 (6C-6E) and V4 (6F-6H) melanoma cells. Data are represented as mean ± SEM (n=3). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (*p<0.05; **p<0.005; ***p<0.0005; ****p<0.0001).

[0020] Figure 7A shows the results of an immunoblot analysis showing expression of LAX1, SIT1 and TRAT1 by CD8⁺ T cells isolated from WT (C57BL/6) mouse spleens after activation for 0, 1, 2, 3 or 4 consecutive days by anti-CD3 and anti-CD28. c-Myc serves as a positive control as it also induced in T cells by anti-CD3+anti-CD28. Beta-actin serves as a loading control.

[0021] Figure 7B shows the results of an immunoblot analysis showing expression of LAX1, SIT1 and TRAT1 by CD8⁺ splenocytes from OT1 TCR transgenic (OT1-tg) mice after activation for 0, 1, 2 or 3 consecutive days by either a high affinity (N4) peptide or low affinity (V4) peptide derived from chicken ovalbumin (OVA). C-Myc and Beta-actin served as positive control or loading control, respectively.

[0022] Figure 8A shows a schematic representation of the vectors used for generation of *Lax1*. Shown is the gene-targeting strategy for the mouse *Lax1* gene in ES cells to generate

Lax1-flox conditional knockout mice. The *Lax1* floxed allele can be deleted by the expression of Cre recombinase (e.g., *dLCK-Cre*).

[0023] Figure 8B shows validation of the gene deletion by Western blot for LAX1 protein. Shown are the results of an immunoblot analysis for LAX1 protein in CD8⁺ spleen cells from *OT1-tg; dLCK-Cre; Lax1* fl/fl mice that had been stimulated with N4-OVA peptide. Beta-actin served as loading control.

[0024] Figure 8C shows a schematic representation of the vectors used for generation of *Trat1*. Shown is the gene-targeting strategy for the mouse *Trat1* gene. *Trat1* was targeted in ES cells to create a *Trat1* floxed conditional knockout allele. The *Trat1* floxed allele can be deleted by the expression of Cre recombinase (e.g., *dLCK-Cre*).

[0025] Figure 8D shows validation of the gene deletion by Western blot for TRAT1 protein. Shown are the results of an immunoblot analysis for TRAT1 protein in CD8⁺ spleen cells from *OT1-tg; dLCK-Cre; Trat1* fl/fl mice stimulated with N4-OVA peptide. Beta-actin served as loading control.

[0026] Figure 8E shows a schematic representation of the vectors used for generation of *Sit1*. Shown is the gene-targeting strategy for the mouse *Sit1* gene. ES cells were targeted with the construct shown to create *Sit1* floxed a conditional knockout allele that can be deleted by Cre recombinase (e.g., *dLCK-Cre*).

[0027] Figure 8F shows validation of the gene deletion by Western blot for SIT1 (8F) protein. Shown are the results of an immunoblot analysis for SIT1 protein in CD8⁺ spleen cells from *OT1-tg; dLCK-Cre; Sit1* fl/fl mice stimulated with N4-OVA peptide. Beta-actin served as loading control.

[0028] Figures 9A-9D are graphs showing the effect of *Lax1* deletion on the growth of B16F10-N4 (high-affinity antigen expressing) tumor cell growth *in vivo*. B16F10 melanoma cells expressing ovalbumin N4-peptide were injected subcutaneously into B6 mice. 7 days later, OT1 TCR transgenic (*OT1-tg*) CD8⁺T cells were injected intraperitoneally into the tumor bearing mice. Three groups of mice were evaluated: mice injected with PBS (untreated) (9B), mice injected with *OT1-tg; dLCK-Cre* CD8⁺ T cells (OT1 only controls) (9C), mice injected with *OT1-tg; dLCK-Cre; Lax1* fl/fl CD8⁺T cells (*OT1; dLCK-Cre; Lax1* fl/fl) lacking LAX1 (9D). Averaged tumor growth curves from all experimental mice are shown in Figure 9A. Data are represented as mean $\pm$ SEM (n=6-7 each group). Student's t-test (unpaired two-tailed) was used to compare between 2 groups and to determine

significance (**** $p < 0.0001$). Individual growth curves from all experimental mice are shown in Figures 9B-9D.

[0029] Figures 10A-10D are graphs showing the effect of *Lax1* deletion on B16F10-V4 (low-affinity antigen expressing) tumor cell growth *in vivo*. B16F10 melanoma cells expressing V4-peptide were injected subcutaneously into B6 mice. 7 days later, OT1 TCR transgenic (*OT1-tg*) CD8⁺ T cells were injected intraperitoneally into the tumor bearing mice. Three groups of mice were evaluated: mice injected with PBS (untreated) (10B), mice injected with *OT1-tg; dLCK-Cre* CD8⁺ T cells (OT1 only controls) (10C), mice injected with *OT1-tg; dLCK-Cre; Lax1 fl/fl* CD8⁺ T cells (*OT1; dLCK-Cre; Lax1 fl/fl*) lacking LAX1 (10D). Averaged tumor growth curves from all experimental mice are shown in Figure 10A. Data are represented as mean $\pm$ SEM (n=7-9 each group). Student's t-test (unpaired two-tailed) was used to compare between 2 groups and to determine significance (** $p < 0.005$; **** $p < 0.0001$). Individual growth curves for all experimental mice are shown in Figures 10B-10D.

[0030] Figures 11A-11B are survival plots of the experiments shown in Figures 9A-9D (Figure 11A) and Figures 10A-10D (Figure 11B). Long-rank (Mantel-cox test) was performed to determine the significance of the results. (** $p < 0.005$; *** $p < 0.0005$; **** $p < 0.0001$).

[0031] Figure 12A is a schematic of the LAX1-based retroviral constructs used for transduction experiments. Y, tyrosine; F, phenylalanine; numbers indicate the amino acid position in human or mouse LAX1.

[0032] Figure 12B-12C show flow cytometry plots showing the expression of LNGFR by retrovirally transduced CD8⁺ OT1 T cells (LNGFR is encoded by the retrovirus and is used as a method for calculating transduction efficiency). *OT1-tg* CD8⁺ spleen T cells were left untransduced or transduced with control (empty) retrovirus (OT1_Vector), LAX1 8F vector (OT1_LAX1-8YF), or LAX1 4F vector (OT1_LAX1-4YF). For comparison, *OT1-tg; dLCK-Cre; Lax1 fl/fl* CD8⁺ T cells were transduced control (empty) vector (*OT1-tg; dLCK-Cre; Lax1 fl/fl*_vector). 5 days after retroviral transduction, transduction efficiency was assessed by surface expression of LNGFR reporter by flow cytometry as shown. Cells were activated by N4-OVA peptide prior to retroviral transduction.

[0033] Figures 13A-13B depict the results of XCELLIGENCE real-time killing assays showing the growth (cell index) of N4 (13A) or V4 (13B) peptide-expressing B16F10 melanoma cells over time after addition of CD8⁺ effector T cells from OT1 TCR-tg mice that

were transduced with the indicated retroviruses depicted in Figure 12A. 10,000 B16F10 cells were plated in each well. Effector T cell: Target melanoma cell ratios used were 10:1, 5:1, 2.5:1, 1.25:1, 0.63:1, 0.31:1 and 0.16:1. TRITON X-100 detergent solution served as a positive control for cell lysis. No effector cells were added for untreated samples. For simplicity only, the results obtained with E:T 0.325:1 are shown for B16F10-N4 melanoma cells (Figure 13A), and only the results obtained with E:T 10:1 are shown for B16F10-V4 melanoma cells (Figure 13B). Data are represented as mean values (n=3). Student's t-test (unpaired two-tailed) was used to determine significance comparing 2 groups (*p<0.05; **p<0.005; ***p<0.0005).

[0034] Figure 14A shows the results of an evaluation of *LAX1* deletion in T cells electroporated with Cas9 plus *LAX1* gRNAs or control (non-specific) gRNAs by Western blot. Three guide RNAs (gRNAs) were used together to delete *LAX1* in human donor PBMC by electroporation with CAS9 enzyme.

[0035] Figure 14B-14E shows the results of an experiment in which human PBMC, which had been electroporated with Cas9 plus control gRNAs (Control CRISPR) or *LAX1* gRNAs (*LAX1* KO CRISPR), were subsequently activated and transduced with retrovirus encoding a human TCR (1G4) specific for the cancer-testis tumor antigen NY-ESO1. Expression of 1G4 TCR was detected with NY-ESO1 tetramer by flow-cytometry. Contour plots show CD4 vs CD8 staining of gated CD3⁺ cells (left panels) or NY-ESO1 tetramer staining of CD8⁺ cells (right panels). Staining with mouse TCRB (PE-mTCRb) detected the transduction efficiency (14C, 14E).

[0036] Figures 15A, 15C, 15E, and 15G depict results of xCELLigence real-time killing assays (RTCA) showing growth (cell index) of human SK-MEL-526 melanoma cells expressing peptides derived from the cancer-testis antigen, NY-ESO1, recognized by the human 1G4 TCR. 1G4 affinity for NY-ESO1 peptides with single amino acid substitutions ranges from High-9V>4A>5Y>8S-Low. 20,000 SK-MEL-526 cells were plated in each well. Effector T cell:Target melanoma cell ratio used: 10:1, 5:1, 2.5:1, 1.25:1, 0.63:1, 0.31:1 and 0.16:1. TRITON X-100 detergent solution served as a positive control for cell lysis. No effector T cells were added for untreated cells. For simplicity only, E:T 0.625:1 and E:T 1.25:1 are shown for SK-MEL-526-9V (Figures 15A) and SK-MEL-526-4A (Figures 15C), only E:T 1.25:1 and 2.5:1 are shown for SK-MEL-526-5Y (Figures 15E) and only E:T 5:1 and 10:1 are shown for SK-MEL-526-8S (Figures 15G). RTCA showed enhanced tumoricidal activity of *LAX1* deleted CD8⁺ T cells compared to control *LAX1*⁺ CD8⁺ T cells

against SK-MEL-526 melanoma cells expressing high affinity (9V), intermediate affinity (4A, 5Y) or low affinity (8S) 1G4 ligands. Data are represented as mean $\pm$ SEM (n=3). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (*p<0.05; **p<0.005; ***p<0.0001).

[0037] Figures 15B, 15D, 15F, and 15H are graphs summarizing the data obtained in Figures 15A, 15C, 15E, and 15G, respectively. Data are summarized at a single timepoint, namely, 48 hrs., and are shown as % cell lysis. Data are represented as mean $\pm$ SEM (n=3). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (*p<0.05; **p<0.005; ***p<0.0001).

[0038] Figure 16 is a graph showing that deletion of *LAX1* in human CD8+ T cells results in increased cytokine TNF α production. Shown is the production of TNF α by 9V peptide stimulated T cells at 24 hr. Cytokines were measured by CBA kit (BD Biosciences). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (ns= not significant; *p<0.05).

[0039] Figure 17 shows the results of a Western blot analysis showing that deletion of *Lax1* enhances the TCR signaling response as exemplified by AKT phosphorylation in CD8+ T cells. WT or *LAX1* deleted CD8+ T cells were activated for 4 consecutive days by anti-CD3 plus anti-CD28 antibody treatment. Western blot shows enhanced activation of AKT (pAKT) in *LAX1* deficient CD8+ T cells. Induction of c-Myc served as a positive control. Blotting for beta-actin served as a loading control.

[0040] Figure 18 shows flow cytometry plots showing that *Sit1* is deleted in T cells from *dLCK-Cre; Sit1 fl/fl* mice. SIT1 expression in CD4+ and CD8+ T cells from control, *dLCK-Cre; Sit1 -/+ (WT)* and *dLCK-Cre; Sit1 fl/fl* mice was detected by intracellular staining with APC fluorochrome conjugated anti-SIT1 antibody and by flow cytometry.

[0041] Figures 19A-19D are graphs showing the effect of T cells lacking *Sit1* on B16F10-N4 (high-affinity OT1 antigen expressing) tumor cell growth *in vivo*. N4-peptide-expressing B16F10 melanoma cells were injected subcutaneously into B6 mice. 7 days later, the indicated OT1+ CD8+ T cells were injected intraperitoneally into the tumor bearing mice. Three groups of mice were evaluated: mice injected with PBS (untreated) (19B), mice injected with *OT1-tg; dLCK-Cre* CD8+ T cells (OT1 only) (19C), or mice injected with *OT1-tg; dLCK-Cre; Sit1 fl/fl* CD8+ T cells (*OT1; dLCK-Cre; Sit1 fl/fl*) (19D). Averaged tumor growth curves are shown in Figure 19A. Data are represented as mean $\pm$ SEM (n=6-7 each group). Student's t-test (unpaired two-tailed) was used to compare between 2 groups

(**** $p < 0.0001$). Individual growth curves for all experimental mice are shown in Figures 19B, 19C and 19D.

[0042] Figures 20A-20D are graphs showing the effect of T cells lacking *Sit1* on B16F10-V4 (low-affinity OT1 antigen-expressing) on tumor cell growth *in vivo*. V4-peptide-expressing B16F10 melanoma cells were injected subcutaneously into B6 mice. 7 days later, the indicated OT1+ CD8+ T cells were injected intraperitoneally into the tumor bearing mice. Three groups of mice were evaluated: mice injected with PBS (untreated) (20B), mice injected with *OT1-tg; dLCK-Cre* CD8+ T cells (OT1 only) (20C), or mice injected with *OT1-tg; dLCK-Cre; Sit1 fl/fl* CD8+ T cells (*OT1; dLCK-Cre; Sit1 fl/fl*) (20D). Averaged tumor growth curves are shown in Figure 20A. Data are represented as mean $\pm$ SEM (n=6-7 each group). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (** $p < 0.05$; *** $p < 0.001$). Individual growth curves for all experimental mice are shown in Figs. 20B, 20C and 20D.

[0043] Figures 21A-21B are survival plots of the experiments shown in Figures 19A-19D (Fig. 21A) and Figures 20A-20D (Fig. 21B). Long-rank (Mantel-Cox test) was performed to analyze significance. (** $p < 0.005$; *** $p < 0.0005$; **** $p < 0.0001$).

[0044] Figure 22A is a schematic of the SIT1-based retroviral constructs used for mouse OT1-tg CD8+ T cell transduction experiments. Y, tyrosine; F, phenylalanine; numbers indicate the amino acid position in human or mouse SIT1.

[0045] Figure 22B-22C show flow cytometry plots showing the expression of LNGFR to assess transduction efficiency on retroviral transduced CD8+ OT1 T cells. CD8+ OT1-tg spleen T cells were left un-transduced (IgG control), transduced with control retrovirus (vector only), or retrovirus encoding SIT1_6Y/6F, SIT1_73F+153F or SIT1_153F. Transduction efficiency was measured 5 days after retroviral transduction by evaluating expression of LNGFR reporter by flow cytometry. Cells were activated by N4-OVA peptide prior to transduction.

[0046] Figures 23A-23F are graphs showing results of XCELLIGENCE real-time killing assays indicating growth (cell index) of N4 (23A, 23B, and 23C) or V4 (23D, 23E, and 23F) peptide-expressing B16F10 melanoma cells over time after addition of CD8+ effector T cells from OT1 TCR-tg mice that were transduced with the indicated retrovirus. 10,000 B16F10 cells were plated in each well. Effector T cell:Target melanoma cell ratios used: 10:1, 5:1, 2.5:1, 1.25:1, 0.63:1, 0.31:1 and 0.16:1. TRITON X-100 detergent solution served as a positive control for cell lysis. No effector cells were added for untreated samples. For

simplicity, E:T 0.625:1 is shown for B16F10-N4 (Figures 23A, 23B, and 23C) and E:T 1.25:1 is shown for B16F10-V4 (Figures 23D, 23E, and 23F) melanoma cells. Data are represented as mean $\pm$ SEM (n=3). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (**p<0.005; ***p<0.0005).

[0047] Figure 24A shows the results of the validation of *SIT1* deletion by intracellular staining and flow cytometry. Three *SIT1* guide RNAs (gRNAs) were electroporated into human donor PBMC together with CAS9 enzyme.

[0048] Figure 24B shows the results of an experiment in which human PBMC, which were rendered *SIT1* deficient by CRISPR/Cas9, were transduced with retrovirus encoding a human TCR (1G4) specific for the cancer-testis tumor antigen NY-ESO1. Expression of 1G4 TCR was detected with NY-ESO1 tetramer by flow-cytometry. Contour plots show CD8 vs NY-ESO1 tetramer staining of CD3+ cells. Staining with mouse TCRB (PE-mTCRb) detected the transduction efficiency.

[0049] Figures 25A, 25C, 25E, and 25G are graphs showing the results of XCELLIGENCE real-time killing assays (RTCA) showing the effect of *SIT1* deletion in 1G4 TCR-transduced T cells on growth (cell index) of human SK-MEL-526 melanoma cells expressing antigenic peptides derived from NY-ESO1. Affinity of single amino acid substitution peptides for the 1G4 TCR ranges from High-9V>4A>5Y>8S-Low. 20,000 SK-MEL-526 cells were plated in each well. Effector T cells were electroporated with either non-specific sgRNAs (Control CRISPR) or *SIT1* sgRNA (SIT1 CRISPR), then transduced with retrovirus encoding 1G4 TCR prior to being added to SK-MEL-526 cultures. Effector T cell: Target melanoma cell ratio used: 10:1, 5:1, 2.5:1, 1.25:1, 0.63:1, 0.31:1 and 0.16:1. TRITON X-100 detergent solution served as a positive control for cell lysis. No effector T cells were added for untreated cells. For simplicity, E:T 1.25:1 and 2.5:1 is shown for SK-MEL-526-9V (Figure 25A), E:T 0.625:1 and 1.25:1 for SK-MEL-526-4A (Figure 25C), E:T 1.25:1 and 2.5:1 for SK-MEL-526-5Y (Figure 25E) and E:T 5:1 and 10:1 for SK-MEL-526-8S (Figure 25G). Notably, RTCA showed enhanced tumoricidal activity of *SIT1* deleted CD8-T cells as compared to control *SIT1*+ CD8+ T cells against SK-MEL-526-9V (High affinity 1G4 ligand expressing) (Figure 25A) as well as SK-MEL-526-8S (Low affinity 1G4 ligand expressing) melanoma cells (Figure 25G). Similar increased tumor cell killing activity was also seen in 4A or 5Y expressing SK-MEL-526 cells (Figures 25C and 25E). Data are represented as mean $\pm$ SEM (n=3). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (**p<0.005; ***p<0.0005; ****p<0.0001).

[0050] Figures 25B, 25D, 25F, and 25H are graphs summarizing the data presented in Figures 25A, 25C, 25E, and 25G, respectively. Data show cell lysis (%) at one time point and E:T ratio for comparison. Data are represented as mean $\pm$ SEM (n=3). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (**p<0.005; ***p<0.0005; ****p<0.0001).

[0051] Figures 26A-26B show that deletion of *SIT1* results in increased cytokine production by human CD8+ T cells. Shown are production of IL-2 (26A) or IFN γ (26B) by T cells 48 hr. after stimulation with 4A NY-ESO1 peptide. Cytokines were measured by CBA kit (BD Biosciences). Student's t-test (unpaired two-tailed) was used to compare T cells electroporated with non-specific sgRNA (sgControl) or *SIT1* sgRNA (sgSIT1), then transduced with 1G4 TCR. ns= not significant, **p<0.005, ****p<0.0001.

[0052] Figure 27A shows contour plots showing perforin and granzyme B expression (% positive cells) in human sgControl or sgSIT1 treated CD8+ T cells that were stimulated with antiCD3+antiCD28. Deletion of *SIT1* increased perforin and granzyme B expression in human CD8+ T cells.

[0053] Figure 27B shows graphs showing % perforin+ granzyme B+ (double positive) CD8+ cells (left) and Mean Fluorescence Intensity (MFI) of granzyme B (GrzB) (center) and perforin (right) in sgControl and sgSIT1 human CD8+ T cells. Data are represented as mean $\pm$ SEM (n=4-5). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (*p<0.05).

[0054] Figures 28A-28D are graphs showing that *Trat1* deletion enhances the ability of mouse OT1+ CD8+ T cells to restrict the growth of N4 (high-affinity), OVA antigen-expressing B16F10 melanoma tumor cells *in vivo*. N4-peptide-expressing B16F10 melanoma cells were injected into B6 mice. 7 days later, CD8+T cells were injected into the tumor bearing mice and tumor size was measured every 4 days. Three groups of mice were evaluated. Untreated mice were injected with PBS (28B), OT1 only mice were injected with *OT1-tg; dLCK-Cre* CD8+T cells (28C), *OT1; dLCK-Cre; Trat1 fl/fl* mice were injected with *OT1-tg; dLCK-Cre; Trat1 fl/fl* CD8+T cells (28D). Figure 28A shows averaged results from all experiments. Data are mean $\pm$ SEM tumor volume (n=6-8 mice in each group). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (***p<0.0005). In Figures 28B-28D, results of all individual experiments are shown.

[0055] Figures 29A-29D are graphs showing that *Trat1* deletion enhances the ability of mouse OT1+ CD8+ T cells to restrict the growth of V4 (low-affinity) OVA antigen

expressing B16F10 melanoma tumor cells *in vivo*. N4-peptide-expressing B16F10 melanoma cells were injected into B6 mice. 7 days later, CD8+T cells were injected into the tumor bearing mice and tumor size was measured every 4 days. Three groups of mice were evaluated. Untreated mice were injected with PBS (29B), OT1 only mice were injected with *OT1-tg; dLCK-Cre* CD8+T cells (29C), *OT1; dLCK-Cre; Trat1 fl/fl* mice were injected with *OT1-tg; dLCK-Cre; Trat1 fl/fl* CD8+T cells (29D). Figure 29A shows averaged results from all experiments. Data are mean $\pm$ SEM tumor volume (n=6-14 mice in each group).

Student's t-test (unpaired two-tailed) was used to compare between 2 groups

(***p<0.0005). In Figures 29B-29D, results of all individual experiments are shown.

[0056] Figures 30A-30B are survival plots of the experiments shown in Figures 28A-28D (Fig. 30A) and Figures 29A-29D (Fig. 30B). Long-rank (Mantel-cox test) was performed to analyze the % of survival. (*p<0.05; ***p<0.0005; ****p<0.0001).

[0057] Figure 31A shows validation of *TRAT1* deletion by intracellular staining and flow cytometry. Two *TRAT1* guide RNAs (gRNAs) were electroporated into human donor PBMC together with CAS9 enzyme.

[0058] Figure 31B shows the results of an experiment in which human PBMC, which had been rendered *TRAT1* deficient by CRISPR/Cas9, were transduced with retrovirus encoding a human TCR (1G4) specific for the cancer-testis tumor antigen NY-ESO1. Expression of 1G4 TCR was detected with NY-ESO1 tetramer by flow-cytometry. Contour plots show CD4 vs 1G4 staining of gated CD3+ cells. Staining with mouse TCRB (PE-mTCRb) detected the transduction efficiency.

[0059] Figures 32A, 32C, and 32E show the results of XCELLIGENCE real-time killing assays (RTCA) showing the effect of *TRAT1* deletion in 1G4 TCR transduced T cells on growth (cell index) of human SK-MEL-526 melanoma cells expressing antigenic peptides derived from NY-ESO1. Affinity of single amino acid substitution peptides for the 1G4 TCR ranges from High-9V>5Y>8S-Low. 20,000 SK-MEL-526 cells were plated in each well. Effector T cells were electroporated with either non-specific sgRNAs (Control CRISPR) or *TRAT1* sgRNA (*TRAT1* CRISPR), then transduced with retrovirus encoding 1G4 TCR prior to being added to SK-MEL-526 cultures. Effector T cell: Target melanoma cell ratio used: 10:1, 5:1, 2.5:1, 1.25:1, 0.63:1, 0.31:1 and 0.16:1. TRITON X-100 detergent solution served as a positive control for cell lysis. No effector T cells were added for untreated cells. For simplicity, E:T 1.25:1 and 2.5:1 is shown for SK-MEL-526-9V (Figure 32A), E:T 5:1 and 10:1 for SK-MEL-526-5Y (Figure 32C) and E:T 5:1 and 10:1 for SK-MEL-526-8S (Figure

32E). Notably, RTCA showed enhanced tumoricidal activity of *TRAT1* deleted CD8-T cells as compared to control *TRAT1*+ CD8+ T cells against SK-MEL-526-9V (High affinity 1G4 ligand expressing) (Figure 32A) as well as SK-MEL-526-5Y (Figure 32C) and SK-MEL-526-8S (Figure 32E), each low affinity 1G4 ligand expressing melanoma cells. Data are represented as mean $\pm$ SEM (n=3). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (*p<0.05; ***p<0.0005).

[0060] Figures 32B, 32D, and 32F are graphs summarizing the data presented in Figures 32A, 32C, and 32E, respectively. Data show cell lysis (%) at one time point and E:T ratio for comparison. Data are represented as mean $\pm$ SEM (n=3). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (*p<0.05; ***p<0.0005).

[0061] Figures 33A-33C are graphs showing that deletion of *TRAT1* in human CD8+ T cells results in increased cytokine production. Shown are production of IFN γ (33A) and TNF α (33B) by 9V peptide stimulated T cells and TNF α production by 8S peptide stimulated T cells at 48hr (33C). Cytokines were measured by CBA kit (BD Biosciences). Student's t-test (unpaired two-tailed) was used to compare between 2 groups (ns= not significant; *p<0.05; **p<0.005).

[0062] Figure 34 is a schematic of TRAT1-based retroviral constructs. Y, tyrosine; F, phenylalanine; numbers indicate the amino acid position in human or mouse TRAT1.

DETAILED DESCRIPTION OF THE INVENTION

Cells, Populations of Cells, Nucleic Acids, Recombinant Expression Vectors, Mutant Proteins, and Pharmaceutical Compositions

[0063] Signaling Threshold Regulating Transmembrane Adaptor 1 (SIT1), Lymphocyte Transmembrane Adaptor 1 (LAX1), and T Cell Receptor Associated Transmembrane Adaptor 1 (TRAT1) are lymphocyte transmembrane adapter proteins (Traps) that were first identified over 20 years ago (Simeoni et al., *Immunol. Rev.*, 224: 215-228 (2008)). Each of these proteins is expressed during T cell development and in mature T cells. SIT1, LAX1 and TRAT1 each have extremely short extracellular domains. To the best of the inventors' knowledge, there have been very few or no publications on SIT1, LAX1 and TRAT1 since their discovery in the early 2000's and no studies have been reported evaluating their potential as targets for immunotherapy.

[0064] It has now been discovered that the deletion, inactivation, or reduction of the activity and/or expression of SIT1, LAX1 or TRAT1 in T cells enhances the reactivity of the T cells against the target antigen.

[0065] An aspect of the invention provides a T cell expressing an antigen-specific receptor, wherein the T cell has been modified to reduce one or both of expression and activity of polypeptide(s) encoded by one or more of SIT1, LAX1 and TRAT1.

[0066] The T cell may express an antigen-specific receptor. The phrases “antigen-specific” and “antigenic specificity,” as used herein, mean that the antigen-specific receptor can specifically bind to and immunologically recognize a target antigen, or an epitope thereof, such that binding of the antigen-specific receptor to the target antigen, or the epitope thereof, elicits an immune response by causing T cell activation. The antigen-specific receptor may be exogenous or endogenous to the cell. By “exogenous” is meant that the antigen-specific receptor is not native to (naturally-occurring on) the cell. By “endogenous” is meant that the antigen-specific receptor is native to (naturally-occurring on) the cell.

[0067] In an aspect of the invention, the antigen-specific receptor is an endogenous T cell antigen receptor (TCR) (for example, in the case of Tumor-Infiltrating-Lymphocytes (TILs)). In some aspects, the T cell comprising the endogenous TCR does not comprise (e.g., express) an exogenous antigen-specific receptor. In other aspects, a T cell comprising an endogenous antigen-specific TCR can also be transformed, e.g., transduced or transfected, with one or more nucleic acids encoding an exogenous (e.g., recombinant) TCR or other recombinant antigen-specific receptor [e.g., Chimeric Antigen Receptor (CAR)]. Such exogenous antigen-specific receptors, e.g., TCRs, can confer specificity for additional antigens to the transformed T cell beyond the antigens for which the endogenous TCR is naturally specific. This can, but need not, result in the production of T cells having dual antigen specificities.

[0068] In an aspect of the invention, the antigen-specific receptor is an exogenous TCR. The exogenous TCR may be a recombinant TCR. A recombinant TCR is a TCR which has been generated through recombinant expression of one or more exogenous TCR α -, β -, γ -, and/or δ -chain encoding genes that assemble with endogenous invariant CD3 chains [CD3 γ (gamma), CD3 δ (delta), CD3 ϵ (epsilon), CD3 ζ (zeta)]. A recombinant TCR can comprise polypeptide chains derived entirely from a single mammalian species, or the recombinant TCR can be a chimeric or hybrid TCR comprised of amino acid sequences derived from TCRs from two different mammalian species. For example, the TCR can comprise a variable region derived from a human TCR, and a constant region of a murine

TCR such that the TCR is “murinized” to promote correct pairing of TCR chains. Any exogenous TCR having antigenic specificity for a target antigen may be useful in the inventive methods and compositions. The TCR generally comprises two polypeptides (i.e., polypeptide chains), such as an α -chain of an $\alpha\beta$ TCR, a β -chain of an $\alpha\beta$ TCR, a γ -chain of a $\gamma\delta$ TCR, a δ -chain of a $\gamma\delta$ TCR, or a combination thereof that are responsible for antigen recognition and that assemble with invariant CD3 signal transducing chains to form a complete TCR complex. Such polypeptide chains of TCRs are known in the art. The antigen-specific TCR can comprise any amino acid sequence, provided that the TCR can specifically bind to and immunologically recognize a target antigen (or epitope thereof). Examples of exogenous TCRs that may be useful in the inventive methods and compositions include, but are not limited to, those disclosed in, for example, U.S. Patents 7,820,174; 7,915,036; 8,088,379; 8,216,565; 8,431,690; 8,613,932; 8,785,601; 9,128,080; 9,345,748; 9,487,573; 9,822,162; 9,879,065; 10,174,098; 11,306,131; and 11,352,410, each of which is incorporated herein by reference.

[0069] In an aspect of the invention, the antigen-specific receptor is a CAR. Typically, a CAR is a single chain receptor that comprises the antigen binding domain of an antibody, e.g., a single-chain variable fragment (scFv), fused to the transmembrane domain of a TCR subunit or a TCR co-receptor and an intracellular domain of a TCR subunit (e.g., CD3zeta). Thus, the antigenic specificity of a TCR can be encoded by a scFv which specifically binds to the target antigen (or epitope thereof). Any CAR having antigenic specificity for a cancer antigen, an autoimmune disease related self-antigen or an infectious disease antigen may be useful in the inventive methods and compositions. Examples of CARs that may be useful in the inventive methods and compositions include, but are not limited to, those disclosed in, for example, U.S. Patents 8,465,743; 9,266,960; 9,765,342; 9,359,447; 9,868,774 and 10,287,350, each of which is incorporated herein by reference.

[0070] In an aspect of the invention, the antigen-specific receptor is a T cell receptor fusion construct (TRUC) (TCR² Therapeutics, Cambridge, MA) (also referred to as T cell receptor fusion proteins). T cell receptor fusion constructs are disclosed in U.S. Patent Application Publication No. 2018/0244747. T cell receptor fusion constructs comprise an antibody-based binding domain that is conjugated or fused to TCR subunit chains. Thus, the antigenic specificity of the T cell receptor fusion construct can be encoded by an scFv which specifically binds to the target antigen (or epitope thereof). The T cell receptor fusion construct includes the endogenous TCR's six CD3 subunit chains. Unlike CARs, T cell

receptor fusion constructs become a functional component of the endogenous TCR complex. Unlike TCRs, T cell receptor fusion constructs bind to antigen independent of the major histocompatibility complex (MHC).

[0071] In an aspect of the invention, the antigen-specific receptor is a bispecific engager TCR fusion protein (e.g., IMMTAC (immune-mobilizing monoclonal TCRs against cancer) molecules). Bispecific engager TCR fusion proteins have two components. One component comprises a soluble TCR. The other component comprises an anti-CD3 effector. The anti-CD3 effector may be any molecule that engages with a CD3 molecule on T cells and activates a T cell immune response. For example, the anti-CD3 effector may be an anti-CD3 antibody or anti-CD3 antibody fragment. The soluble TCR component of the bispecific engager TCR fusion protein binds to the target antigen presented on the surface of cancer cells presented by an HLA molecule. The anti-CD3 effector component engages a CD3 molecule on T cells. The engagement of these components of the bispecific engager TCR fusion protein triggers the activation and recruitment of T cells and redirects T-cell killing to tumor cells.

[0072] In an aspect of the invention, the antigen-specific receptor is a T cell antigen coupler (TAC). A TAC has three components: (1) an antigen-binding domain, (2) a TCR-recruitment domain, and (3) a co-receptor domain (hinge, transmembrane, and cytosolic regions). The antigen binding domain binds to a target antigen. For example, the antigen binding domain may be an scFv that binds to the target antigen. The TCR-recruitment domain interacts with and co-opts the endogenous TCR expressed by the cell. For example, the TCR-recruitment domain may be an anti-CD3 scFv that binds to the CD3 of the TCR complex expressed by the cell. The co-receptor domain anchors the TAC in the cell membrane and either activates or silences the T cell depending on the presence of the target antigen. The co-receptor domain may include, for example, the CD4 hinge, CD4 transmembrane (TM) region, and CD4 intracellular region.

[0073] In an aspect of the invention, the antigen-specific receptor is a synthetic TCR and antigen receptor (STAR). A STAR is a double-chain TCR $\alpha\beta$ -based receptor with variable regions of immunoglobulin heavy and light chains (V_H and V_L) fused to the TCR alpha chain constant region and the TCR beta chain constant region, respectively.

[0074] In an aspect of the invention, the antigen-specific receptor has antigenic specificity for a target antigen. The target antigen may be a cancer antigen, an autoimmune disease related self-antigen, or an infectious disease antigen.

[0075] In an aspect of the invention, the antigen-specific receptor has antigenic specificity for a cancer antigen. The term “cancer antigen,” as used herein, refers to any molecule (e.g., protein, polypeptide, peptide, lipid, carbohydrate, etc.) solely or predominantly expressed or over-expressed by a tumor cell or cancer cell, such that the antigen is associated with the tumor or cancer. The cancer antigen can additionally be expressed by normal, non-tumor, or non-cancerous cells. However, in such cases, the expression of the cancer antigen by normal, non-tumor, or non-cancerous cells is usually not as robust as the expression by tumor or cancer cells. In this regard, the tumor or cancer cells can over-express the antigen or express the antigen at a significantly higher level, as compared to the expression of the antigen by normal, non-tumor, or non-cancerous cells. Also, the cancer antigen can additionally be expressed by cells of a different state of development or maturation. For instance, the cancer antigen can be additionally expressed by cells of the embryonic or fetal stage, which cells are not normally found in an adult host. Alternatively, the cancer antigen can be additionally expressed by stem cells or precursor cells, which cells are not normally found in an adult host. Examples of cancer antigens include, but are not limited to, mesothelin, CD19, CD22, CD30, CD70, CD276 (B7H3), gp100, MART-1, Epidermal Growth Factor Receptor Variant III (EGFRVIII), Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2), TRP-1, TRP-2, tyrosinase, human papillomavirus (HPV) 16 E6, HPV 16 E7, HPV 18 E6, HPV 18 E7, KK-LC-1, NY-BR-1, NY-ESO-1 (also known as CAG-3), SSX-2, SSX-3, SSX-4, SSX-5, SSX-9, SSX-10, MAGE-A1, MAGE-A2, BRCA, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, HER-2, etc. In an aspect of the invention, the cancer antigen may be a mutated antigen that is expressed or overexpressed by tumor or cancer cells and which is not expressed by normal, non-tumor, or non-cancerous cells. Examples of such cancer antigens may include, but are not limited to, mutated KRAS and mutated p53. T cells having antigenic specificity for a cancer antigen may, advantageously, reduce or avoid cross-reactivity with normal tissues such as, for example, that which may occur using T cells having antigenic specificity for minor histocompatibility antigens. In a preferred aspect, the cancer antigen is encoded by an infecting virus (HPV 16 E7, HPV 16 E6, HPV 18 E7, HPV 18 E6), or is a Cancer Testis Antigen (i.e., multifunctional proteins that are specifically expressed in male spermatozoa and tumor cells but not in healthy somatic cells; e.g., MAGE, BAGE, KK-LC-1). In an aspect of the invention, the

cancer antigen may be a non-mutated cancer antigen (e.g., self-cancer peptide) or cancer neo-antigen.

[0076] The cancer antigen can be an antigen expressed by any cell of any cancer or tumor, including the cancers and tumors described herein. The cancer antigen may be a cancer antigen of only one type of cancer or tumor, such that the cancer antigen is associated with or characteristic of only one type of cancer or tumor. Alternatively, the cancer antigen may be a cancer antigen (e.g., may be characteristic) of more than one type of cancer or tumor. For example, the cancer antigen may be expressed by both breast and prostate cancer cells and not expressed at all by normal, non-tumor, or non-cancer cells.

[0077] The term “infectious disease antigen” as used herein refers to any molecule (e.g., protein, peptide, lipid, carbohydrate, etc.) solely or predominantly expressed an agent that can cause an infection that can lead to a disease, such that the antigen is associated with the agent. Such agents may include, for example, bacteria, viruses, fungi, and parasites. Accordingly, the infectious disease antigen may be a bacterial antigen, viral antigen, fungal antigen, or parasite antigen.

[0078] Examples of viral antigens may include, but are not limited to, those expressed by herpes viruses, pox viruses, hepadnaviruses, papilloma viruses, adenoviruses, coronaviruses, orthomyxoviruses, paramyxoviruses, flaviviruses, and caliciviruses. Further examples of viral antigens may include, but are not limited to, respiratory syncytial virus (RSV), influenza virus, herpes simplex virus, Epstein-Barr virus, human immunodeficiency virus, varicella virus, cytomegalovirus, hepatitis A virus, hepatitis B virus, hepatitis C virus, human T-lymphotropic virus, calicivirus, adenovirus, and Arena virus. Viral antigens are known in the art and include, for example, any viral protein, e.g., env, gag, pol, gp120, thymidine kinase, and the like. In an aspect of the invention, the viral antigen may one associated with a cancer-causing virus such as, for example, HPV 16 E6, HPV 16 E7, HPV 18 E6, or HPV 18 E7.

[0079] Examples of bacterial antigens may include, but are not limited to, those expressed by a bacteria selected from the group consisting of *Streptococcus pneumoniae*, *Neisseria Meningitides*, *Haemophilus influenzae*, *Streptococcus agalactiae*, *Listeria monocytogenes*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Ureaplasma urealyticum*, *Moraxella catarrhalis*, *Clostridium perfringens*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Helicobacter pylori*, *Campylobacter jejuni*, *Salmonella enterica*, *Enterococcus faecalis*, *Clostridium difficile*,

Staphylococcus saprophyticus, *Treponema pallidum*, *Haemophilus ducreyi*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila*.

[0080] Examples of fungal antigens may include, but are not limited to, those expressed by *Cryptococcus*, *Aspergillus*, *Coccidioides*, *Histoplasma*, *Blastomyces*, and *Pneumocystis*.

[0081] The parasitic antigen may, for example, be an antigen expressed by any of the three main classes of parasites that can cause disease in humans: protozoa, helminths, and ectoparasites. Examples of parasitic antigens may include, but are not limited to, Sarcodina (e.g., *Entamoeba*), Mastigophora (e.g., *Giardia*, *Leishmania*), Ciliophora (*Balantidium*), Sporozoa (e.g., *Plasmodium*, *Cryptosporidium*), flatworms (platyhelminths) (e.g., trematodes (flukes) and cestodes (tapeworms), and roundworms (nematodes).

[0082] “Autoimmune disease related self-antigen,” as used herein, refers to any protein, peptide, enzyme complex, ribonucleoprotein complex, and post-translationally modified antigen normally expressed by an individual and against which the individual’s T cells are directed.

[0083] In an aspect of the invention, the cell which has been modified is a T-cell. For purposes herein, the T cell can be any T cell, e.g., a primary T cell or a T cell obtained from a mammal, for example, a human or a mouse. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to blood, bone marrow, lymph node, the thymus, bone marrow, or other tissues or fluids. T cells can also be enriched for or purified. Preferably, the T cell is a human T cell. More preferably, the T cell is a T cell isolated from a human. In some cases, as in the case of TILs, antigen-specific T cells may be isolated, enriched for or purified from a tumor biopsy. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to CD4⁺ helper T cell, e.g., Th₁ and Th₂ cell, CD4⁺ T cells, CD8⁺ T cell (e.g., cytotoxic T cell), tumor infiltrating lymphocyte (TIL), memory T cell (e.g., central memory T cell and effector memory T cell), naïve T cells, regulatory T cell (Treg), natural killer T (NKT) cell, mucosal-associated invariant T (MAIT) cell, an invariant natural killer T (iNKT) cell, gamma delta T cell ($\gamma\delta$ T cells), or alpha beta ($\alpha\beta$) T cell, and the like. In another aspect of the invention, the T cell is derived from an induced pluripotent stem cell (iPSC), embryonic stem cell or hematopoietic stem cell. In other aspect, the T cell is derived from a hematopoietic stem cell.

[0084] In an aspect of the invention, the T cell has been modified to reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1*. The biological activity of SIT1, LAX1 or TRAT1 may be inhibited in any manner, e.g., by inhibiting the expression of

the polypeptide and/or by inhibiting SIT1, LAX1 or TRAT1 signaling, as compared to that which is observed in a corresponding wild-type T cell. The biological activity may be inhibited to any degree that realizes a beneficial therapeutic effect. For example, in some aspects, the biological activity may be completely inhibited (i.e., prevented), while in other aspects, the biological activity may be partially inhibited (i.e., reduced).

[0085] For example, the T cell may be modified to express one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1*. For example, the mutant protein may be a “dominant negative” form of SIT1, LAX1 or TRAT1 that competes with the endogenous, wild-type SIT1, LAX1 or TRAT1, thereby preventing or reducing the biological activity of the wild-type proteins. The biological activity of SIT1, LAX1 or TRAT1 may be reduced or prevented in any manner, e.g., by reducing or preventing SIT1, LAX1 or TRAT1 signaling, as compared to that which is observed in the absence of the corresponding mutant protein.

[0086] Although they have not been extensively studied, early data suggest that SIT1, LAX1 and TRAT1 function to inhibit signaling in T cells initiated by the TCR (Simeoni et al., *supra*). SIT1, LAX1 and TRAT1 each have extremely short extracellular domains, so they are thought to act by tonically inhibiting TCR signaling, perhaps by localizing with the TCR, rather than through interaction with a specific ligand. Each of SIT1, LAX1 and TRAT1 contains conserved tyrosines within their cytoplasmic domain that are known or thought to interact with cytosolic proteins that regulate TCR signaling, including the inhibitory protein tyrosine phosphatase SHP2 (PTPN11), Phosphatidylinositol-3 kinase (PI3K), and the adapter proteins GRB2 and GADS (Fig. 1 of Simeoni et al., *supra*). The biological activity of wild-type SIT1, LAX1 or TRAT1 may be reduced or prevented, e.g., by reducing or preventing the interaction of SIT1, LAX1 or TRAT1 with one or more of PTPN11, PI3K, GRB2 and GADS; by reducing or preventing the biological activity of one or more of PTPN11, PI3K, GRB2 and GADS; by reducing or preventing the inhibition of signaling in T cells initiated by the TCR; and/or by reducing or preventing the localization of endogenous SIT1, LAX1 or TRAT1 with the TCR.

[0087] In an aspect of the invention, the one or more mutant proteins comprise one or more of truncated SIT1, truncated LAX1 and truncated TRAT1. For example, the truncated version of SIT1, LAX1 or TRAT1 may retain the extracellular and transmembrane domain and lack all or part of the cytoplasmic domain. The amino acid residue position numbers of the extracellular domain, transmembrane domain and cytoplasmic domain of human SIT1,

human LAX1 (isoform a) and human TRAT1 (isoform 1) are set forth in Table 1. Nucleotide and amino acid sequences of wild-type human SIT1, human LAX1 and human TRAT1 are set forth in Table 2. The part of the cytoplasmic domain that is lacking in the truncated version may be, for example, that portion of the cytoplasmic domain that provides the biological activity of SIT1, LAX1 or TRAT1. In an aspect of the invention, the truncated version lacks 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 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the N-terminal or C-terminal amino acid residues of the cytoplasmic domain.

TABLE 1

	Human SIT1 (SEQ ID NO: 2)	Human LAX1 Isoform a (SEQ ID NO: 8)	Human TRAT1 Isoform 1 (SEQ ID NO: 12)
Extracellular domain	Amino acid residue position numbers 1-40	Amino acid residue position numbers 1-37	Amino acid residue position numbers 1-7
Transmembrane domain	Amino acid residue position numbers 41-61	Amino acid residue position numbers 38-58	Amino acid residue position numbers 8-28
Cytoplasmic domain	Amino acid residue positions 62-196	Amino acid residue positions 59-398	Amino acid residue positions 29-186

TABLE 2

Protein	Sequence
Human <i>SIT1</i> transcript sequence GenBank Accession No: NM_014450.3	AGTATTGGGGATGCTGAGCTGCGGGGTACGGGCCTGAGGAGG GATGGGAGTAAGAAGTGCTGTGGAAACCGTCAGGCCATGAAC CAGGCTGACCCTCGGCTCAGAGCAGTGTGCTTGTGGACTCTC ACATCTGCAGCCATGAGCAGAGGCGACAACCTGCACGGATCTA CTCGCACTGGGAATCCCCTCCATAAACCAGGCCTGGGGACTG TGGTCTCTCTTAGGGGCTGTGACGCTGCTATTTCTCATCTCGC TGGCTGCACACTTGTCCCAGTGGACCAGGGGCCGGAGCAGGA GCCATCCGGGGCAGGGACGCTCTGGAGAGTCTGTGGAAGAGG TCCCGCTGTATGGGAACCTGCATTATCTACAGACAGGACGGC TGTCTCAAGACCCAGAGCCAGACCAGCAGGATCCAACCTTTG GAGGCCCTGCCAGGGCTGCAGAGGAGGTGATGTGCTATACCA GCCTGCAGCTGCGGCCTCCTCAGGGTCGGATCCCCGGTCTTG GAACCCCCGTCAAGTACTCGGAGGTGGTGTGCTGGACTCTGAGC CAAAGTCCCAGGCCTCGGGCCCCGAGCCGGAGCTCTATGCCT CAGTATGTGCCAGACCCGCAGGGCCCCGGGCCTCCTTCCCGG

Protein	Sequence
	ATCAGGCCTATGCCAACAGCCAGCCTGCAGCCAGCTGAGATG GAGGGCCTGGCACAGCGGGGCGTGCACTGCCCCAGCCCCCG TAGCAGGGGCATGACTGTTTCCCAACCAGCACCCAAAGACGG GCGCCATTGCCAAGTCACAGGATGTGATCTACCCCGGACTTC CTATCTGAGCTTCAAGGGAGACATCTCAGGGCAAAGCTTTCG TGATGGAGGAGGCAAAGACAGTAGCCCCCTCCTTATTTCTTTT TTCTATCTGTTCTCTTAGCCCCCAAACCTCCAGGTTCTCACTT CCTTCTTCTGGAGTTTAACCAGATCCTCCCCACCCCGCTCCC TCATAGTCTACCCCCACGCCTCAGTGTCTCCTCAGGCACAGGA AGTGGGCGGTGGGGGAGGGGTAAGGGCCTGACAGTGGGTGG GTGGGTATATTCCTCAGGAGTCCACAGACTGGAGTGGACCTG GAACTTAGAGACGGGAGGGACCCGAGCCTGGCTTTTGACCTA AGAACCTAGCAGGAGAATACAGTCTCCATCCTGCTGTCTCT GTCCTGTCCCCAAGTTTCAAATAAACTTTCCAAAAAGTG (SEQ ID NO: 1)
Human SIT1 protein sequence GenBank Accession No: NP_055265.1	MNQADPRLRAVCLWLTSAAMSRGDNCTDLLALGIPSITQAWG LWVLLGAVTLLFLISLAHLSQWTRGRSRSHPGQGRSGESVEEV PLYGNLHYLQTGRLSQDPEPDQDPTLGGPARAAEEVMCYTSL QLRPPQGRIPGPGTPVKYSEVVLDSKPSQASGPEPELYASVCAQ TRRARASFPDQAYANSQPAAS (SEQ ID NO: 2)
Human <i>LAX1</i> transcript variant 2 NM_00113619 0.2	ACTCTGCTTTGGGTGTTGAAGGAAGACGAGCTTCTCACGGAG CCTCTCTTGAGCCTCTTGGCAGTTTCCCCCTCTGTGCCCTCAC GTTTCCACCAGAAACGTGAAGGCAGAGGCCACAGATTCTCCC TGAGCCACCTCACTTGGAAGCACCATGTCCGGATGAGATCGC ACTTCCTGCAGTGGGCATTAGCCACGTCCAGAAATAAAGACC AGATCACCAACATCTTTTCCGGGTTTGCGGGACTCCTCGCCAT CCTCCTGGTTCGTTGCGGTTTTCTGCATCTTGTGGAATTGGAAT AAACGGAAGAAGCGACAAGTTCCTTACCTCCGAGTTACCGTC ATGCCCTTGCTGACTTTGCCACAAACCAGACAAAGAGCCAAA AATATTTATGACATCTTGCCTTGGCGACAGGAAGACCTGGGG AGACATGAGTCGAGGAGTATGCGCATTTTCAGTACTGAGAGC CTCCTCTCCAGAAATTCTGAGAGCCCCGAGCATGTGCCCTCCC AAGCAGGCAATGCCTTCCAGGAGCATAACAGCCACATCCATG CCACAGAGTACGCGGTGGGTATCTATGACAACGCCATGGTCC CCCAGATGTGTGGGAACCTCACTCCCTCGGCACACTGCATCA ATGTCAGAGCTTCCAGAGACTGCGCAAGCATTTCTTCAGAGG ATTGCGCATGATTATGTCAATGTCCCCACAGCAGAAGAGATTG CTGAGACTCTAGCTTCTACCAAAAGCCCTTCCAGAAATCTCTT TGTCTTCCCAGTACCCAGAAGCTGGAGTTTACTGAGGAAAG AGATGAGGGCTGTGGAGATGCTGGTGACTGCACCAGTTTGTA TTCTCCAGGAGCTGAGGACAGTGATTCACTCAGCAATGGAGA AGGTTCTTCTCAGATCTCAAATGACTATGTCAACATGACAGG GTTGGATCTCAGTGCCATCCAGGAAAGGCAGCTCTGGGTGGC TTTTCAGTGCTGCAGAGACTATGAAAATGTTCCAGCAGCAGA TCCCAGTGGAAGCCAGCAGCAGGCTGAGAAAGATGTGCCATC CTCAAACATAGGTCATGTGAGGACAAGACAGATGATCCCGG GACCCATGTCCAATGTGTCAAAAGGACATTCCTTGCTTCAGG

Protein	Sequence
	GGATTATGCAGACTTTCAGCCATTACACAGAGTGAGGACAG TCAGATGAAACATAGAGAAGAGATGTCAAATGAGGACTCCA GTGACTATGAAAATGTGCTAACTGCCAAGTTAGGAGGCAGGG ACTCTGAGCAGGGGCTGGCACTCAGCTCCTTCCTGATGAAT GAAGACCCAGGTACCCAGCCATAAAGCCACATTGAGTAGTCT ATCCCATAGGATTGACTACTGCAGAGTCTAGTGCAGACCCGT GATCACCTTAGTGCTTCAGTGGATTCACTGGTTAGATTA AAAA GAGGCTGAGATGAGCAGTGAAC TAAGAGGCCACACAAAAGC AGAGGTTTGGGAATTCCAGAAGGGAATTCCTCTCAAGCAGAG TGTGGTTATCTCTGTACCAGCCTAAGAATGTTTGCTGAAACT GCTTCCTAGAACTGTGAAGAAAGCAGGAAAGTAGTGCACAGT AGTCTAAGATTATTACCTTCATTAATACCAACAGGCTGCAAA GCAAGAGTATAGATTATTGTATAATCCAGTCAGAGGTCAAAA GGAAGGAAGAAGTTGGAGTGGAGTGGGGTGGGCAATTTCCAT TTTAAAGAGTGTAGGCAGGCCAGGTGCAGTGGCTCATGCCTG TAATCCCAGCATTTTGGTAGGCCGAGGAGGGCAGATCACTTG GTGCCAGGAGTTCTAGACCAGCCTGGCCAACATGACCCATCT TACTAAAAATACAAAAATTAGCCAGGCGTGGTGGTGTGCCC CTGTAATCCCAGCTACTCAGGAGGCTGAAGCAGGAGAATCGC TTGAACCCGGGAGGTGGAGGTTGCAGCAAGCCGAGATTGTGC CACTGTACTCCAGCCTGGTGACAGAGCGAGACTCCCTCTCAA AAAAAAAAAACAAACAAAAAGAAGTGTGGGCAACCAAATGT AGGTAAGGATGAAGAAGAACAGGAACCTCACTGGTGGCTG ATGGGATTGCAAAGCGTCAGGGCAGCTTTGAAAACTTTTCA GCAGTTTCTTATAAGCATAACTTATCATATGGCCCAGCCATTT CACTACTAGAAATTGACGCAAGTGAAC TGAACCTTATGTTT ACACAAAACTCGCACATGACTGTTTATAGCAGCTTCATTCAT AATTACCAAAAACTAGAAATAACCCAGATATCCTTCAACAGG TGATTGGATAAGCTGTTGATATCCATTCCTTGAGTTTATCCAT ACTCAACAGTAAAAATGAACCTTGGTTCACACAATAATAT AGACATATCTTAAATCCATTTTGCTAAGTGAAAGAAGCTAGG CCTAAAAGTCTACATGTTGGGCGATTCTATTTATATGACATTC TGGAAGAGGCAAACTATAGGGATGAAGAACAGATCAGTTG TTGTCAGGTTTGAGAAAAGGAGGGAAGTTTGCTACAAAGGGG CTGCAAAGGGAATAGGCTGATGGAGCTGTTCTGTATGGTACT GTGTGGTGGATACATGACAATATCCATTTGCAAACACAAGAA CTATACACATGGCCAGGCGCGGTGGCTCACACCCGTAATCCC AGCACTTTGCGGGGCCGAGGCAGGGGAACACAAGGTCAGGA GATCAAGACTGTCCTGGACAACATGGTGAAACCCGTGTCTCTA CTGTCTCTAACATTTGTAAACCTGTCTCTAATTTGTAATTTGTA AACCCTGTCTCTCATATTTGTAAACTACAAAAATTAGCTTGG CATGGTGGTGTGCGCCTGTAGTCCCAGCTACTAGGAGGCTGA GGCAGGAGAATCACTTGAACCAGGAAGGCGGACTGCATTCCA GCCTGGGCAACAGAGAAAGACTCTGTCTCAAAAAAAAAAAAA AAAAAAAAAA (SEQ ID NO: 3)
Human <i>LAX1</i> transcript variant 3	GCATTTCAACACCCAAGCAAAACCGAGCAGTGACGCCAGCCC TGCCAGGGAATTGCAAGAAATAAAGACCAGATCACCAACATC TTTTCCGGGTTTGCGGGACTCCTCGCCATCCTCCTGGTCGTTG

Protein	Sequence
NM_00128287 8.1	CGGTTTTCTGCATCTTGTGGAATTGGAATAAACGGAAGAAGC GACAAGTTCCTTACCTCCGAGTTACCGTCATGCCCTTGCTGAC TTTGCCACAAACCAGACAAAGAGCCAAAAATATTTATGACAT CTTGCCTTGGCGACAGGAAGACCTGGGGAGACATGAGTCGAG GAGTATGCGCATTTTCAGTACTGAGAGCCTCCTCTCCAGAAAT TCTGAGAGCCCCGAGCATGTGCCCTCCCAAGCAGGCAATGCC TTCCAGGAGCATACAGCCCACATCCATGCCACAGAGTACGCG GTGGGTATCTATGACAACGCCATGGTCCCCAGATGTGTGGG AACCTCACTCCCTCGGCACACTGCATCAATGTCAGAGCTTCCA GAGACTGCGCAAGCATTTCCTCAGAGGATTTCGCATGATTATGT CAATGTCCCCACAGCAGAAGAGATTGCTGAGACTCTAGCTTC TACCAAAGCCCTTCCAGAAATCTCTTTGTTCTTCCCAGTACC CAGAAGCTGGAGTTTACTGAGGAAAGAGATGAGGGCTGTGG AGATGCTGGTGACTGCACCAGTTTGTATTCTCCAGGAGCTGA GGACAGTGATTCACTCAGCAATGGAGAAGGTTCTTCTCAGAT CTCAAATGACTATGTCAACATGACAGGGTTGGATCTCAGTGC CATCCAGGAAAGGCAGCTCTGGGTGGCTTTTCAGTGCTGCAG AGACTATGAAAATGTTCCAGCAGCAGATCCCAGTGGAAGCCA GCAGCAGGCTGAGAAAGATGTGCCATCCTCAAACATAGGTCA TGTCGAGGACAAGACAGATGATCCCGGGACCCATGTCCAATG TGTCAAAAGGACATTCTTGCTTCAGGGGATTATGCAGACTTT CAGCCATTACACAGAGTGAGGACAGTCAGATGAAACATAGA GAAGAGATGTCAAATGAGGACTCCAGTGACTATGAAAATGTG CTAACTGCCAAGTTAGGAGGCAGGGACTCTGAGCAGGGGCCCT GGCACTCAGCTCCTTCCTGATGAATGAAGACCCAGGTACCCA GCCATAAAGCCACATTGAGTAGTCTATCCCATAGGATTGACT ACTGCAGAGTCTAGTGCAGACCCGTGATCACCTTAGTGCTTCA GTGGATTCACTGGTTAGATTAAAAAGAGGCTGAGATGAGCAG TGAACATAAGAGGCCACACAAAAGCAGAGGTTTGGAATTCCA GAAGGGAATTCTTCTCAAGCAGAGTGTTGTTATCTCCTGTACC AGCCTAAGAATGTTTGCTGAAACTGCTTCCTAGAACTGTGAA GAAAGCAGGAAAGTAGTGCACAGTAGTCTAAGATTATTACCT TCATTAATACCAACAGGCTGCAAAGCAAGAGTATAGATTATT GTATAATCCAGTCAGAGGTCAAAGGAAGGAAGAAGTTGGA GTGGAGTGGGGTGGGCAATTTCCATTTTAAAGAGTGTAGGCA GGCCAGGTGCAGTGCTCATGCCTGTAATCCCAGCATTTTGGT AGGCCGAGGAGGGCAGATCACTTGGTGCCAGGAGTTCTAGAC CAGCCTGGCCAACATGACCCATCTTTACTAAAAATACAAAAA TTAGCCAGGCGTGGTGGTGTGCCCCTGTAATCCCAGCTACTCA GGAGGCTGAAGCAGGAGAATCGCTTGAACCCGGGAGGTGGA GGTTGCAGCAAGCCGAGATTGTGCCACTGTACTCCAGCCTGG TGACAGAGCGAGACTCCCTCTCAAAAAAAAAAAAAACAAACAAA AAAGAAAGTGTGGGCAACCAATGTAGGTAAGGATGAAGAAG AACAGGAACCTCACTGGTGGCTGATGGGATTGCAAAGCGTC AGGGCAGCTTTGAAAACTTTTCAGCAGTTTCTTATAAGCATA ACTTATCATATGGCCCAGCCATTTCACTACTAGAAATTGACGC AAGTGAAC TGAAAAC TTATGTTACACAAAAA ACTCGCACATG ACTGTTTATAGCAGCTTCATTCATAATTACCAAAAACTAGAAA

Protein	Sequence
	TAACCCAGATATCCTTCAACAGGTGATTGGATAAGCTGTTGAT ATCCATTCCCTTGAGTTTATCCATACTCAACAGTAAAAAATGAA CTCTTGGTTCACACAATAATATAGACATATCTTAAATCCATTT TGCTAAGTGAAAGAAGCTAGGCCTAAAAGTCTACATGTTGGG CGATTCTATTTATATGACATTCTGGAAAAGGCCAAAATATAG GGATGAAGAACAGATCAGTTGTTGTCAGGTTTGAGAAAAGGA GGGAAGTTTGCTACAAAGGGGCTGCAAAGGGAATAGGCTGAT GGAGCTGTTCTGTATGGTACTGTGTGGTGGATACATGACAAT ATCCATTTGCAAACACAAGAACTATACACATGGCCAGGCGCG GTGGCTCACACCCGTAATCCCAGCACTTTGCGGGGCCGAGGC AGGGGAACACAAGGTCAGGAGATCAAGACTGTCCTGGACAA CATGGTGAAACCCTGTCTCTACTGTCTCTAACATTTGTAAACC TGTCTCTAATTTGTAATTTGTAAACCCTGTCTCTCATATTTGTA AAACTACAAAAATTAGCTTGGCATGGTGGTGTGCGCCTGTAG TCCCAGCTACTAGGAGGCTGAGGCAGGAGAATCACTTGAACC AGGAAGGCGGACTGCATTCCAGCCTGGGCAACAGAGAAAAGA CTCTGTCTCAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 4)
Human <i>LAX1</i> transcript variant 1 NM_017773.4	ACTCTGCTTTGGGTGTTGAAGGAAGACGAGCTTCTCACGGAG CCTCTCTTGAGCCTCTTGGCAGTTTCCCCCTCTGTGCCCTCAC GTTTCCACCAGAAACGTGAAGGCAGAGGCCACAGATTCTCCC TGAGCCACCTCACTTGGAAGCACCATGTCCGGATGAGATCGC ACTTCCTGCAGTGGGCATTAGCCACGTCCAGGTAGAACCAAA CCTGTTGCTTTTGTATGTTGGGTCAACTTGGCCTGACGTTTCA GAGGTAGACACGAGATAGGGAGTTTGTGCGGGGGTGGGGA GAAGTGGTAGACATGCTGGCTAACTGATTATGATTAAGAGAA ACTTAGAAGCTGAAGCCAGAGAGCATCTCAAAGGTTCTCTGAT ACAATGGATGGTGTCACTCCAACCCTTTGACAATCAGAGGG AGGACCTTGGAGTCCAGCACTCTGCATGTGACTCCCCGCAGC CTGGACAGAAATAAAGACCAGATCACCAACATCTTTTCCGGG TTTGCGGGACTCCTCGCCATCCTCCTGGTCGTTGCGGTTTTCT GCATCTTGTGGAATTGGAATAAACGGAAGAAGCGACAAGTTC CTTACCTCCGAGTTACCGTCATGCCCTTGCTGACTTTGCCACA AACCAGACAAAGAGCCAAAAATATTTATGACATCTTGCCCTTG GCGACAGGAAGACCTGGGGAGACATGAGTCGAGGAGTATGC GCATTTTCAGTACTGAGAGCCTCCTCTCCAGAAATTCTGAGAG CCCGGAGCATGTGCCCTCCCAAGCAGGCAATGCCTTCCAGGA GCATACAGCCCACATCCATGCCACAGAGTACGCGGTGGGTAT CTATGACAACGCCATGGTCCCCCAGATGTGTGGGAACCTCAC TCCCTCGGCACACTGCATCAATGTCAGAGCTTCCAGAGACTG CGCAAGCATTTCTTCAGAGGATTTCGCATGATTATGTCAATGTC CCCACAGCAGAAGAGATTGCTGAGACTCTAGCTTCTACCAAA AGCCCTTCCAGAAATCTCTTTGTTCTTCCCAGTACCCAGAAGC TGGAGTTTACTGAGGAAAGAGATGAGGGCTGTGGAGATGCTG GTGACTGCACCAGTTTGTATTCTCCAGGAGCTGAGGACAGTG ATTCACTCAGCAATGGAGAAGGTTCTTCTCAGATCTCAAATG ACTATGTCAACATGACAGGGTTGGATCTCAGTGCCATCCAGG AAAGGCAGCTCTGGGTGGCTTTTCAGTGCTGCAGAGACTATG AAAATGTTCCAGCAGCAGATCCCAGTGGAAGCCAGCAGCAGG

Protein	Sequence
	CTGAGAAAGATGTGCCATCCTCAAACATAGGTCATGTGCGAGG ACAAGACAGATGATCCCGGGACCCATGTCCAATGTGTCAAAA GGACATTCTTGCTTCAGGGGATTATGCAGACTTTCAGCCATT CACACAGAGTGAGGACAGTCAGATGAAACATAGAGAAGAGA TGTCAAATGAGGACTCCAGTGACTATGAAAATGTGCTAACTG CCAAGTTAGGAGGCAGGGACTCTGAGCAGGGGCCTGGCACTC AGCTCCTTCCTGATGAATGAAGACCCAGGTACCCAGCCATAA AGCCACATTGAGTAGTCTATCCCATAGGATTGACTACTGCAG AGTCTAGTGCAGACCCGTGATCACCTTAGTGCTTCAGTGGATT CACTGGTTAGATTAAAAAGAGGCTGAGATGAGCAGTGAACATA AGAGGCCACACAAAAGCAGAGGTTTGGGAATTCCAGAAGGG AATTCTTCTCAAGCAGAGTGTGGTTATCTCCTGTACCAGCCTA AGAATGTTTGCTGAAACTGCTTCCTAGAACTGTGAAGAAAGC AGGAAAGTAGTGCACAGTAGTCTAAGATTATTACCTTCATTA ATACCAACAGGCTGCAAAGCAAGAGTATAGATTATTGTATAA TCCAGTCAGAGGTCAAAAGGAAGGAAGAAAGTTGGAGTGGAG TGGGGTGGGCAATTTCCATTTTAAAGAGTGTAGGCAGGCCAG GTGCAGTGGCTCATGCCTGTAATCCCAGCATTTTGGTAGGCCG AGGAGGGCAGATCACTTGGTGCCAGGAGTTCTAGACCAGCCT GGCCAACATGACCCATCTTTACTAAAAATACAAAATTAGCC AGGCGTGGTGGTGTGCCCTGTAATCCCAGCTACTCAGGAGG CTGAAGCAGGAGAATCGCTTGAACCCGGGAGGTGGAGGTTGC AGCAAGCCGAGATTGTGCCACTGTACTCCAGCCTGGTGACAG AGCGAGACTCCCTCTCAAAAAAAAAAAAAACAAAACAAAAAGAA GTGTGGGCAACCAAATGTAGGTAAGGATGAAGAAGAACAGG AACTCTCACTGGTGGCTGATGGGATTGCAAAGCGTCAGGGCA GCTTTGAAAACTTTTCAGCAGTTTCTTATAAGCATAAATTAT CATATGGCCCAGCCATTTCACTACTAGAAATTGACGCAAGTG AACTGAAAACTTATGTTACACAAAACTCGCACATGACTGT TTATAGCAGCTTCATTCATAATTACCAAAAACTAGAAATAAC CCAGATATCCTTCAACAGGTGATTGGATAAGCTGTTGATATCC ATTCCTTGAGTTTATCCATACTCAACAGTAAAAAATGAACCTCT TGGTTCACACAATAATATAGACATATCTTAAATCCATTTTGCT AAGTGAAAGAAGCTAGGCCTAAAAGTCTACATGTTGGGCGAT TCTATTTATATGACATTCTGGAAAAGGCAAAACTATAGGGAT GAAGAACAGATCAGTTGTTGTCAGGTTTGAGAAAAGGAGGGA AGTTTGCTACAAAGGGGCTGCAAAGGGAATAGGCTGATGGAG CTGTTCTGTATGGTACTGTGTGGTGGATACATGACAATATCCA TTTGCAAACACAAGAACTATACACATGGCCAGGCGCGGTGGC TCACACCCGTAATCCCAGCACTTTGCGGGGCCGAGGCAGGGG AACACAAGGTCAGGAGATCAAGACTGTCCTGGACAACATGGT GAAACCTGTCTCTACTGTCTCTAACATTTGTAAACCTGTCTC TAATTTGTAATTTGTAAACCCTGTCTCTCATATTTGTAAAACT ACAAAAATTAGCTTGGCATGGTGGTGTGCGCCTGTAGTCCCA GCTACTAGGAGGCTGAGGCAGGAGAATCACTTGAACCAGGA AGGCGGACTGCATTCCAGCCTGGGCAACAGAGAAAGACTCTG TCTCAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 5)

Protein	Sequence
Human LAX1 protein transcript variant 2 (isoform b) NP_001129662.1	MRSNFLQWALATSRNKDQITNIFSGFAGLLAILLVAVFCILWNWNKRKKRQVPYLRVTVMPLLTLPQTRQRAKNIYDILPWRQEDLGRHESRSMRIFSTESLLSRNSESPEHVPSQAGNAFQEHTAHIHATEYAVGIYDNAMVPMQCGNLTPSAHCINVRASRDCASISSEDSHDYVNVPTAEEIAETLASTKSPSRNLFVLPSTQKLEFTEERDEGCGDAGDCTSLYSPGAEDSDSLNNEGSSQISNDYVNMTGLDLSAIQERQLWVAFQCCRDYENVPAADPSGSQQQA EKDVPSSNIGHVEDKTD DPGTHVQCVRKRTFLASGDYADFQPFTQSEDSQMKHREEMSNE DSSDYENVLTAKLGGRDSEQGPGTQLLPDE (SEQ ID NO: 6)
Human LAX1 protein transcript variant 3 (isoform c) NP_001269807.1	MPLLTLPQTRQRAKNIYDILPWRQEDLGRHESRSMRIFSTESLLSRNSESPEHVPSQAGNAFQEHTAHIHATEYAVGIYDNAMVPMQCGNLTPSAHCINVRASRDCASISSEDSHDYVNVPTAEEIAETLASTKSPSRNLFVLPSTQKLEFTEERDEGCGDAGDCTSLYSPGAEDSDSLNNEGSSQISNDYVNMTGLDLSAIQERQLWVAFQCCRDYENVPAADPSGSQQQA EKDVPSSNIGHVEDKTD DPGTHVQCVRKRTFLASGDYADFQPFTQSEDSQMKHREEMSNE DSSDYENVLTAKLGGRDSEQGPGTQLLPDE (SEQ ID NO: 7)
Human LAX1 protein transcript variant 1 (isoform a) and NP_060243.2	MDGVPTPLSTIRGRTLESSTLHVTPRSLDRNKDQITNIFSGFAGLLAILLVAVFCILWNWNKRKKRQVPYLRVTVMPLLTLPQTRQRAKNIYDILPWRQEDLGRHESRSMRIFSTESLLSRNSESPEHVPSQAGNAFQEHTAHIHATEYAVGIYDNAMVPMQCGNLTPSAHCINVRASRDCASISSEDSHDYVNVPTAEEIAETLASTKSPSRNLFVLPSTQKLEFTEERDEGCGDAGDCTSLYSPGAEDSDSLNNEGSSQISNDYVNMTGLDLSAIQERQLWVAFQCCRDYENVPAADPSGSQQQA EKDVPSSNIGHVEDKTD DPGTHVQCVRKRTFLASGDYADFQPFTQSEDSQMKHREEMSNE DSSDYENVLTAKLGGRDSEQGPGTQLLPDE (SEQ ID NO: 8)
Human <i>TRAF1</i> transcript variant 2 NM_001317747.2	GGCACAGATAAAGATAAGTTTTACTGTCATGCTGCTTTTAACA TAACAGAGCAACATCACCTAGGAAAAAAGTTTGTAGGAGGAT TTTTAATCCATATATTTGTCTTATGGCTAGATAAAGATTCTCT GAAAAAAGAAGCATGTCAGATAAAATGTACAGCTACTCCAG TGACCACACCAGGGTTGATGAGTATTATATTGAAGACACACC AATTTATGGTAACCTAGATGATATGATTTTCAGAACCAATGGAT GAAAATTGCTATGAACAAATGAAAGCCCGACCAGAGAAATCT GTAAATAAGATGCAGGAAGCCACCCCATCTGCACAGGCAACC AATGAAACACAGATGTGCTACGCCTCACTTGATCACAGCGTT AAGGGGAAGCGTAGAAAGCCAGGAAACAGAATACTCATTT CTCAGACAAGGATGGAGATGAGCAACTACATGCAATAGATGC CAGCGTTTCTAAGACCACCTTAGTAGACAGTTTCTCCCCAGAA AGCCAGGCAGTAGAGGAAAACATTCATGATGATCCCATCAGA CTGTTTGGATTGATCCGTGCTAAGAGAGAACCTATAAACTAG CTGGACCATGATCTAGTTCAATGATTTGGCTCCTATTGAAGAT GGCTTCTAAGAAAACAAGATGCACAGAGGACACAGAAGGAC TTGGCAGCAGGGTGATGACCTGATCATTTGTTGATGGGATGG TGGCTTACCTCTTATTCACAGCTTACACTTATGCATGCCAAAT GTAAGGCCATGAAAATCAGTATTTCAAATAACTTAAAAAATG CTTTACTACTAAAAATGTAAAAAATTAATGTGCTCACCTCGGCA GCACATATACTAAAAATTAATAAGACCCAGCTTGAAAATTGA

Protein	Sequence
	GCCTGATAACAAGATTACAAATTCACAATACCTAATACTTAG GGAAATATAAAAATTTAAGCATGAATGTGTTCTGGAACACGT TAGAAGAAAAATAAAAGCCAATGAGTTTTTTTTTAATTCTCCT TTCTCACCAATGGGCAATAGCCCATAAATTGAAATAAATTTCTG ATTGAAAGGTATAGGAAACATTAAAAATGCATTACTAAGAGAA GTAATATAATTTTCTTACAAAGTATTTTTCCCAAAGATAGCTT TACTATTTCAAAAATTGTCAAATTAATGCATGCTCCTTACAAC AAACAAATATCAAAAAGAGTTTAGGAATTCTACTAGCCAGAG ATAGTCACCTGGAGAACTTTCTATATATCCTTCTAAATATTT TTCTGGGCATGCTTATGTATGTACATCAGTTGTTTCTTTTTATT TTGAACCAAAAATGTGGTTTCTTTTGTACACATTACTTAAACT TTCTTTCCAGTCAACAATATATTGTGGATTTATTTTCACTGTTA TATTTAACTATATATAAATACGCATATATTGTAATTTTAATGT CTGCTTAGCACCCCACTGATAACCAAATCACAGTTTATTTAAA TAATTTTAATGACTTTTCAAAAACAATTTATTGATGCAAAAAG CAAGGTTGAGATGACAATGTTTCTTTCAATAATTAaaaaata CTGCTTCACTGTCTTCTCTCTGTTTCATCAGAAACACATACTAT AATACTTGTCTCTGTCCTTAAGTGTGATACACGCTATTTTCTG ATTGTTAAGACTTTCCTTATTACTGGTTCTAATTTGATTATAAT ATGCCAAGGTATGGT (SEQ ID NO: 9)
Human <i>TRAF1</i> transcript variant 1 NM_016388.4	GGCACAGATAAAGATAAGTTTTACTGTCATGCTGCTTTTAACA TAACAGAGCAACATCACCTAGGAAAAAAGTTTGTAGGAGGAT TTTTAATCCATATATTTGTCTTATGGCTAGATAAAGATTTCTCT GAAAAAAGAAGCATGTCTAGGAATCTCTGGGTGCCCTTTTT CCTCTGGGGACTTCTAGCATTGTTGGGCTTGGCTTTGGTTATA TCACTGATCTTCAATATTTCCCACTATGTGGAAGCAACGAC AAGATAAAATGTACAGCTACTCCAGTGACCACACCAGGGTTG ATGAGTATTATATTGAAGACACACCAATTTATGGTAACTTAG ATGATATGATTTTCAAGAACCAATGGATGAAAATTGCTATGAAC AAATGAAAGCCCCGACCAGAGAAATCTGTAAATAAGATGCAG GAAGCCACCCCATCTGCACAGGCAACCAATGAAACACAGATG TGCTACGCCTCACTTGATCACAGCGTTAAGGGGAAGCGTAGA AAGCCCAGGAAACAGAATACTCATTCTCAGACAAGGATGGA GATGAGCAACTACATGCAATAGATGCCAGCGTTTCTAAGACC ACCTTAGTAGACAGTTTCTCCCCAGAAAGCCAGGCAGTAGAG GAAAACATTCATGATGATCCCATCAGACTGTTTGGATTGATCC GTGCTAAGAGAGAACCTATAAACTAGCTGGACCATGATCTAG TTCAATGATTTGGCTCCTATTGAAGATGGCTTCTAAGAAAACA AGATGCACAGAGGACACAGAAGGACTTGGCAGCAGGGTGAT GACCTGATCATTGTTGATGGGATGGTGGCTTACCTCTTATTC ACAGCTTACACTTATGCATGCCAAATGTAAGGCCATGAAAAT CAGTATTTCAAATAACTTAAAAAATGCTTTACTACTAAAAATGT AAAAAATTAATGTGCTCACCTCGGCAGCACATATACTAAAAA TTAATAAGACCCAGCTTGAAAATTGAGCTGATAACAAGATT ACAAATTCACAATACCTAATACTTAGGGAAATATAAAAATTT AAGCATGAATGTGTTCTGGAACACGTTAGAAGAAAAATAAAA GCCAATGAGTTTTTTTTTAATTCTCCTTTCTCACCATTGGCA ATAGCCCATAATTGAAATAAATTTCTGATTGAAAGGTATAGG

Protein	Sequence
	AAACATTAAAATGCATTACTAAGAGAAGTAATATAATTTTCTT ACAAAGTATTTTCCCAAAGATAGCTTTACTATTTCAAAAATT GTCAAATTAATGCATGCTCCTTACAACAAACAAATATCAAAA AGAGTTTAGGAATTCTACTAGCCAGAGATAGTCACTTGGAGA AACTTTCTATATATCCTTCTAAATATTTTCTGGGCATGCTTAT GTATGTACATCAGTTGTTTCTTTTATTTTGAACCAAAAATGT GGTTTCTTTTGTACACATTACTTAAACTTTCTTTCCAGTCAACA ATATATTGTGGATTTATTTTCACTGTTATATTTAACTATATATA AATACGCATATATTGTAATTTTAATGTCTGCTTAGCACCCAC TGATAACCAAATCACAGTTTATTTAAATAATTTTAATGACTTT TCAAAAACAATTTATTGATGCAAAAAGCAAGGTTGAGATGAC AATGTTTCTTTCAATAATTAATAAATACTGCTTCACTGTCTTC TCTCTGTTTCATCAGAAACACATACTATAATACTTGTCTCTGT CCTTAAGTGTGATACACGCTATTTTCTGATTGTTAAGACTTT CCTTATTACTGGTTCTAATTTGATTATAATATGCCAAGGTATG GT (SEQ ID NO: 10)
Human TRAT1 protein isoform 2 NP_001304676. 1	MSDKMYSYSSDHTRVDEYYIEDTPIYGNLDDMISEPMDENCYEQ MKARPEKSVNKMQEATPSAQATNETQMCYASLDHSVKGKRRK PRKQNTHFSDKDGDEQLHAIDASVSKTTLVDSFSPESQAVEENIH DDPIRLFGLIRAKREPIN (SEQ ID NO: 11)
Human TRAT1 protein isoform 1 NP_057472.2	MSGISGCPFFLWGLLALLGLALVISLIFNISHYVEKQRQDKMYSY SSDHTRVDEYYIEDTPIYGNLDDMISEPMDENCYEQMKARPEKS VNKMQEATPSAQATNETQMCYASLDHSVKGKRRKPRKQNTHF SDKDGDEQLHAIDASVSKTTLVDSFSPESQAVEENIHDDPIRLFG LIRAKREPIN (SEQ ID NO: 12)

[0088] In an aspect of the invention, the one or more mutant proteins comprise one or more of: (i) a fusion protein comprising (a) a SIT1 extracellular domain, (b) a SIT1 transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s); (ii) a fusion protein comprising (a) a LAX1 extracellular domain, (b) a LAX1 transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s); and (iii) a fusion protein comprising (a) a TRAT1 extracellular domain, (b) a TRAT1 transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s). These fusion proteins may comprise truncated SIT1, truncated LAX1, or truncated TRAT1 (that is, these fusion proteins may lack all or part of the cytoplasmic domain of SIT1, LAX1, or TRAT1), as described herein with respect to other aspects of the invention. Examples of TCR intracellular signaling domains may include, but are not limited to, the intracellular TCR signaling domain of any one of the following proteins: a 4-1BB protein, a CD27 protein, a CD28 protein, a CD8-alpha protein, a CD40 protein, a CD40L protein, an Icos protein, an OX40 protein, an immunoreceptor

tyrosine-based activation motif (ITAM), a CD3gamma protein, a CD3delta protein, a CD3epsilon protein, a CD3zeta protein, MyD88, or any combination of the foregoing. Examples of T cell co-receptor intracellular signaling domains include, but are not limited to, the intracellular signaling domain of either of the following proteins: a CD4 protein, a CD8 protein, or a combination of the foregoing.

[0089] In an aspect of the invention, the one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1* comprise one or more of:

(i) a human SIT1 amino acid sequence, wherein the tyrosine at one or both of positions 90 and 168, as defined by reference to SEQ ID NO: 2 is, independently, substituted with another amino acid;

(ii) a human TRAT1 amino acid sequence, wherein the tyrosine at one or more of positions 43, 45, 79, and 110, as defined by reference to SEQ ID NO: 12 is, independently, substituted with another amino acid; and

(iii) a human LAX1 amino acid sequence, wherein the tyrosine at five or more of positions 71, 93, 150, 155, 193, 268, 294, and 373, as defined by reference to SEQ ID NO: 8 is, independently, substituted with another amino acid. In an aspect of the invention, the tyrosine at one or more of the foregoing positions is, independently, substituted with alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, or valine. In an aspect of the invention, the tyrosine at one or more of the foregoing positions is substituted with phenylalanine.

[0090] Amino acid substitutions of SIT1, TRAT1, and LAX1 are defined herein by reference to the amino acid sequence of SEQ ID NO: 2 (wild-type human SIT1), SEQ ID NO: 12 (wild type human TRAT1 isoform 1), and SEQ ID NO: 8 (wild type human LAX1 isoform a), respectively. When the positions are as defined by SEQ ID NO: 2, 12, or 8, then the actual positions of the amino acid sequence of a particular embodiment of SIT1, TRAT1, and LAX1 are defined relative to the corresponding positions of SEQ ID NO: 2, 12, or 8, respectively, and may represent different residue position numbers than the residue position numbers of SEQ ID NO: 2, 12, or 8, respectively. For example, human LAX1 has three isoforms, which have different N-terminal leader peptide amino acid sequences, but otherwise have identical amino acid sequences. Similarly, human TRAT1 has two isoforms, which have different N-terminal leader peptide amino acid sequences, but otherwise have

identical amino acid sequences. Thus, for example, substitutions refer to a replacement of an amino acid residue in the amino acid sequence of a particular embodiment of human LAX1 corresponding to the indicated position of the 398-amino acid sequence of SEQ ID NO: 8 with the understanding that the actual positions in the respective amino acid sequences in the three human LAX1 isoforms may be different. For example, when the positions are as defined by SEQ ID NO: 8, the reference to position 71 refers to the tyrosine normally present at position 71 of SEQ ID NO: 8, although the position of this tyrosine in the other two isoforms of human LAX1 may be different. In the event of multiple substitutions at two or more positions, the two or more substitutions may be the same or different, i.e., each amino acid residue of the two or more amino acid residues being substituted can be substituted with the same or different amino acid residue unless explicitly indicated otherwise.

[0091] In an aspect of the invention, the T cell is modified to comprise a nucleic acid comprising a nucleotide sequence encoding an RNA interference (RNAi) agent that reduces one or both of expression and activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1*. The RNAi agent can be a nucleic acid at least about 10 nucleotides in length that specifically binds to and is complementary to a target nucleic acid encoding any one or more of *SIT1*, *LAX1* and *TRAT1* or a complement thereof. The RNAi agent may be introduced into the T cell, wherein the T cell is capable of expressing any one or more of *SIT1*, *LAX1* and *TRAT1*, in an effective amount for a time and under conditions sufficient to interfere with expression of any one or more of *SIT1*, *LAX1* and *TRAT1*, respectively. In an aspect, the RNAi agent may comprise a small interfering RNA (siRNA), a short hairpin miRNA (shMIR), a microRNA (miRNA), or an antisense nucleic acid. The RNAi agent, e.g., siRNA, shRNA, miRNA, and/or antisense nucleic acid can comprise overhangs. That is, not all nucleotides need bind to the target sequence. RNA interference nucleic acids employed can be at least about 19, at least about 40, at least about 60, at least about 80, at least about 100, at least about 120, at least about 140, at least about 160, at least about 180, at least about 200, at least about 220, at least about 240, from about 19 to about 250, from about 40 to about 240, from about 60 to about 220, from about 80 to about 200, from about 60 to about 180, from about 80 to about 160, and/or from about 100 to about 140 nucleotides in length.

[0092] The RNAi agent, e.g., siRNA or shRNA, can be encoded by a nucleotide sequence included in a cassette, e.g., a larger nucleic acid construct such as an appropriate vector. Examples of such vectors include, but are not limited to, lentiviral and adenoviral vectors.

When present as part of a larger nucleic acid construct, the resulting nucleic acid can be longer than the comprised RNAi nucleic acid, e.g., greater than about 70 nucleotides in length. In some aspects, the RNAi agent employed cleaves the target mRNA. In other aspects, the RNAi agent employed does not cleave the target mRNA.

[0093] Any type of suitable siRNA, miRNA, and/or antisense nucleic acid can be employed. In an aspect, the antisense nucleic acid comprises a nucleotide sequence complementary to at least about 8, at least about 15, at least about 19, or from about 19 to about 22 nucleotides of a nucleic acid encoding any one or more of *SIT1*, *LAX1* and *TRAT1* or a complement thereof. In an aspect, the siRNA may comprise, e.g., trans-acting siRNAs (tasiRNAs) and/or repeat-associated siRNAs (rasiRNAs). In another aspect, the miRNA may comprise, e.g., a short hairpin miRNA (shMIR).

[0094] In an aspect of the invention, the RNAi agent may inhibit or downregulate to some degree the expression of the protein encoded by the *SIT1*, *LAX1* or *TRAT1* gene, as compared to that which is observed in the absence of the RNAi agent. In this regard, a T cell comprising a nucleotide sequence encoding the RNAi agent expresses none of any one or more of *SIT1*, *LAX1* and *TRAT1* or lower levels of any one or more of *SIT1*, *LAX1* and *TRAT1* as compared to a T cell that lacks the nucleotide sequence encoding the RNAi agent.

[0095] In an aspect, the *SIT1* sequence is a human *SIT1* sequence. For example, human *SIT1* is assigned Gene NCBI Entrez Gene ID No. 27240. The human *SIT1* gene is found on chromosome 9 at 9p13.3. The human *SIT1* transcript sequence includes mRNA GenBank Accession No: NM_014450.3 (SEQ ID NO: 1), with corresponding protein sequence GenBank Accession No: NP_055265.1 (SEQ ID NO: 2). Human genomic *SIT1* sequences include GenBank Accession Nos: NC_000009.12, NC_060933.1, AJ271888.1, and CH471071.2. Human *SIT1* mRNA sequences also include Genbank Accession Nos: AA310964.1, AA746595.1, AJ010059.1, AK314758.1, BC102029.1, BC104491.1, BC107484.1, BM919490.1, and JQ924063.1. Human *SIT1* amino acid sequences include Genbank Accession Nos: CAC81313.1, EAW58370.1, CAB41504.1, BAG37296.1, AAI02030.1, AAI04492.1, AAI07485.1, and AFK32776.1. Other human sequences, as well as other *SIT1* species can be employed in accordance with aspects of the invention.

[0096] In an aspect, the *LAX1* sequence is a human *LAX1* sequence. For example, human *LAX1* is assigned Gene NCBI Entrez Gene ID No. 54900. The human *LAX1* gene is found on chromosome 1 at 1q32.1. Three human transcript variants include mRNA GenBank Accession Nos: NM_001136190.2 (SEQ ID NO: 3) (transcript variant 2), NM_001282878.1

(SEQ ID NO: 4) (transcript variant 3), and NM_017773.4 (SEQ ID NO: 5) (transcript variant 1), with corresponding protein sequences GenBank Accession Nos: NP_001129662.1 (SEQ ID NO: 6) (isoform b), NP_001269807.1 (SEQ ID NO: 7) (isoform c), and NP_060243.2 (SEQ ID NO: 8) (isoform a), respectively. Human genomic *LAX1* sequences include GenBank Accession Nos: NC_000001.11, CH471067.1, and NC_060925.1. Human *LAX1* mRNA sequences also include Genbank Accession Nos: AK000347.1, AK225331.1, AK301076.1, AK301421.1, AY090784.1, BC069650.1, BC089408.1, and DC414389.1. Human *LAX1* amino acid sequences include Genbank Accession Nos: BAA91101.1, BAG62682.1, BAH13480.1, AAM09818.1, AAH69650.1, and AAH89408.1. Other human sequences, as well as other *LAX1* species can be employed in accordance with aspects of the invention.

[0097] In an aspect, the *TRAT1* sequence is a human *TRAT1* sequence. For example, human *TRAT1* is assigned Gene NCBI Entrez Gene ID No. 50852. The human *TRAT1* gene is found on chromosome 3 at 3q13.13. Two human transcript variants include mRNA GenBank Accession Nos: NM_001317747.2 (SEQ ID NO: 9) (transcript variant 2) and NM_016388.4 (SEQ ID NO: 10) (transcript variant 1), with corresponding protein sequences GenBank Accession Nos: NP_001304676.1 (SEQ ID NO: 11) (isoform 2) and NP_057472.2 (SEQ ID NO: 12) (isoform 1), respectively. Human genomic *TRAT1* sequences include GenBank Accession Nos: NC_000003.12, NC_060927.1, AJ240084.1, CH471052.2, and KF457667.1. Human *TRAT1* mRNA sequences also include Genbank Accession Nos: AF161547.1, AJ224878.1, AJ240085.1, AK291991.1, AV735609.1, BC025713.1, BX111605.1, BX329167.2, and BX415345.2. Human *TRAT1* amino acid sequences include Genbank Accession Nos: EAW79717.1, EAW79718.1, AAF29034.1, CAA12178.1, CAB72134.1, BAF84680.1, and AAH25713.1. Other human sequences, as well as other *TRAT1* species can be employed in accordance with aspects of the invention.

[0098] In accordance with an aspect of the invention, the RNAi agent, such as a shMIR, can target a nucleotide sequence selected from the group consisting of the 5' untranslated region (5' UTR), the 3' untranslated region (3' UTR), and the coding sequence of *SIT1*, *LAX1* or *TRAT1*, complements thereof, and any combination thereof. Any suitable *SIT1*, *LAX1* or *TRAT1* target sequence can be employed. In an aspect of the invention, the sequence of the RNAi agent can be designed against all or part of the human *SIT1* sequence of Genbank Accession No. NM_014450.3 (SEQ ID NO: 1). In an aspect of the invention, the sequence of the RNAi agent can be designed against all or part of any one of the human *LAX1* sequences

of GenBank Accession Nos: NM_001136190.2 (SEQ ID NO: 3), NM_001282878.1 (SEQ ID NO: 4), and NM_017773.4 (SEQ ID NO: 5), but also recognize either of the other two *LAX1* sequences. In an aspect of the invention, the sequence of the RNAi agent can be designed against all or part of the human *TRAT1* sequence of Genbank Accession No. NM_001317747.2 (SEQ ID NO: 9) but also recognize the sequence of Genbank Accession No. NM_016388.4 (SEQ ID NO: 10) (or vice-versa). RNAi agents can be designed against any appropriate *SIT1*, *LAX1* or *TRAT1* mRNA sequence.

[0099] In an aspect of the invention, the T cell is modified to comprise an inactivating mutation of (i) gene encoding or (ii) a promoter sequence regulating expression of a gene encoding one or more of *SIT1*, *LAX1* and *TRAT1*. The inactivating mutation may be any one or more mutation(s) that reduces or eliminates (i) the expression of the mRNA or protein encoded by the gene and/or (ii) the biological activity of the protein encoded by the gene. The expression and/or biological activity of the mRNA or protein may be reduced by 10% or more, such as by 20% or more, 30% or more, 40% or more, 50% or more, 80% or more, 70% or more, 80% or more, 90% or more, 95% or more, 97% or more or 99% or more, relative to the protein expressed by the wild-type (non-mutated) gene. In an aspect of the invention, the inactivating mutation may completely eliminate expression and/or biological activity of *SIT1*, *LAX1* or *TRAT1* protein or mRNA. The inactivating mutation may be any type of mutation, for example, a missense, substitution, insertion, or deletion mutation. In an aspect of the invention, the inactivating mutation comprises a deletion or all or part of the (i) gene encoding or (ii) a promoter sequence regulating expression of a gene encoding one or more of *SIT1*, *LAX1* and *TRAT1*.

[0100] Another aspect of the invention provides a nucleic acid comprising a nucleotide sequence encoding one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1*. The mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1* may be as described herein with respect to other aspects of the invention. For example, the one or more mutant proteins encoded by the nucleic acid may comprise one or more of truncated *SIT1*, truncated *LAX1* and truncated *TRAT1*. In another aspect of the invention, the one or more mutant proteins encoded by the nucleic acid may comprise one or more of: (i) a fusion protein comprising (a) a *SIT1* extracellular domain, (b) a *SIT1* transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s); (ii) a fusion protein comprising (a) a *LAX1* extracellular domain, (b) a *LAX1* transmembrane domain,

and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s); and (iii) a fusion protein comprising (a) a TRAT1 extracellular domain, (b) a TRAT1 transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s). These fusion proteins may comprise truncated SIT1, truncated LAX1, or truncated TRAT1 (that is, these fusion proteins may lack all or part of the cytoplasmic domain of SIT1, LAX1, or TRAT1), as described herein with respect to other aspects of the invention. The TCR and/or T cell co-receptor intracellular signaling domains may be as described herein with respect to other aspects of the invention.

[0101] In an aspect of the invention, the one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1* comprise one or more of:

(i) a human SIT1 amino acid sequence, wherein the tyrosine at one or both of positions 90 and 168, as defined by reference to SEQ ID NO: 2 is, independently, substituted with another amino acid;

(ii) a human TRAT1 amino acid sequence, wherein the tyrosine at one or more of positions 43, 45, 79, and 110, as defined by reference to SEQ ID NO: 12 is, independently, substituted with another amino acid; and

(iii) a human LAX1 amino acid sequence, wherein the tyrosine at five or more of positions 71, 93, 150, 155, 193, 268, 294, and 373, as defined by reference to SEQ ID NO: 8 is, independently, substituted with another amino acid. In an aspect of the invention, the tyrosine at one or more of the foregoing positions is, independently, substituted with alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, or valine. In an aspect of the invention, the tyrosine at one or more of the foregoing positions is substituted with phenylalanine.

[0102] “Nucleic acid,” as used herein, includes “polynucleotide,” “oligonucleotide,” and “nucleic acid molecule,” and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide.

[0103] The nucleic acids may be recombinant. As used herein, the term “recombinant” refers to (i) molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above. For purposes herein, the replication can be *in vitro* replication or *in vivo* replication.

[0104] The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. See, for example, Green and Sambrook, *Molecular Cloning: A Laboratory Manual*, 4th ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (2012). For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (e.g., phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, β -D-galactosylqueosine, inosine, N⁶-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N⁶-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, β -D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N⁶-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids of aspects of the invention can be purchased from any of a variety of commercial entities.

[0105] In an aspect of the invention, any of the nucleic acids described herein may comprise a codon-optimized nucleotide sequence. Without being bound to any particular theory or mechanism, it is believed that codon optimization of the nucleotide sequence increases the translation efficiency of the mRNA transcripts. Codon optimization of the nucleotide sequence may involve substituting a native codon for another codon that encodes the same amino acid, but can be translated by tRNA that is more readily available within a cell, thus increasing translation efficiency. Optimization of the nucleotide sequence may also

reduce secondary mRNA structures that would interfere with translation, thus increasing translation efficiency.

[0106] The nucleic acids of aspects of the invention can be incorporated into a recombinant expression vector. In this regard, an aspect of the invention provides a recombinant expression vector comprising any of the nucleic acids described herein.

[0107] For purposes herein, the term “recombinant expression vector” means a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide, or peptide expressed within the cell. The vectors of the invention are not naturally-occurring as a whole. However, parts of the vectors can be naturally-occurring. The inventive recombinant expression vectors can comprise any type of nucleotide, including, but not limited to DNA and RNA, which can be single-stranded or double-stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The recombinant expression vectors can comprise naturally-occurring, non-naturally-occurring internucleotide linkages, or both types of linkages. Preferably, the non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder the transcription or replication of the vector.

[0108] The recombinant expression vector of the invention can be any suitable recombinant expression vector, and can be used to transform or transfect any suitable host cell. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be selected from the group consisting of the transposon/transposase series, pUC series (Fermentas Life Sciences), the pBluescript series (Stratagene, LaJolla, CA), the pET series (Novagen, Madison, WI), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, CA). Bacteriophage vectors, such as λ GT10, λ GT11, λ ZapII (Stratagene), λ EMBL4, and λ NM1149, also can be used. Examples of animal expression vectors include pEUK-Cl, pMAM and pMAMneo (Clontech). Preferably, the recombinant expression vector is a transposon or a viral vector, e.g., a lentiviral vector, a retroviral vector (e.g., MSCV) or an adenovirus vector.

[0109] The recombinant expression vectors of aspects of the invention can be prepared using standard recombinant DNA techniques described in, for example, Green and Sambrook

et al., *supra*. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell.

Replication systems can be derived, e.g., from ColEI, 2 μ plasmid, λ , SV40, bovine papillomavirus, and the like.

[0110] Desirably, the recombinant expression vector comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host cell (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate and taking into consideration whether the vector is DNA- or RNA-based.

[0111] The recombinant expression vector can include one or more selection genes, which allow for selection of transformed or transfected host cells. Selection genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host cell to provide prototrophy, and the like. Suitable selection genes for the inventive expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes. The recombinant expression vector can also include one or more reporter genes which allow for estimation of transduction or transformation efficiency. A commonly used reporter gene for transduction of human T cells is human *LNGFR* which encodes Low affinity Nerve Growth Factor Receptor, a cell surface protein that can be detected by flow cytometry. *LNGFR* was included in each of the retroviral vectors used in the experiments shown in Figs. 4A-4J, 5A-5D, and 6A-6H.

[0112] The recombinant expression vector can comprise a native or nonnative promoter operably linked to the nucleotide sequence encoding the one or more mutant proteins, or to the nucleotide sequence which is complementary to the nucleotide sequence encoding the one or more mutant proteins. The selection of promoters, e.g., strong, weak, inducible, tissue-specific and developmental-specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral promoter, e.g., a human elongation factor-1 α promoter, or a viral promoter, e.g., a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, and a promoter found in the long-terminal repeat of the murine stem cell virus.

[0113] The inventive recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

[0114] Further, the recombinant expression vectors can be made to include a suicide gene. As used herein, the term “suicide gene” refers to a gene that causes the cell expressing the suicide gene to die. The suicide gene can be a gene that confers sensitivity to an agent, e.g., a drug, upon the cell in which the gene is expressed, and causes the cell to die when the cell is contacted with or exposed to the agent. Suicide genes are known in the art and include, for example, the Herpes Simplex Virus (HSV) thymidine kinase (TK) gene, cytosine deaminase, purine nucleoside phosphorylase, and nitroreductase.

[0115] Another aspect of the invention further provides a host cell comprising any of the nucleic acids or any of the recombinant expression vectors described herein. As used herein, the term “host cell” refers to any type of cell that can contain the inventive recombinant expression vector. The host cell can be a eukaryotic cell, e.g., plant, animal, fungi, or algae, or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DH5 α *E. coli* cells, Chinese hamster ovarian cells, monkey VERO cells, COS cells, HEK293 cells, and the like. For purposes of amplifying or replicating the recombinant expression vector, the host cell is preferably a prokaryotic cell, e.g., a DH5 α cell. For purposes of producing a recombinant mutant protein, the host cell is preferably a mammalian cell. Most preferably, the host cell is a human cell. For example, the host cell may be a human lymphocyte. In an aspect of the invention, the host cell is selected from the group consisting of a T cell, a natural killer (NK) cell, a macrophage, a pluripotent cell, and a multipotent cell. While the host cell can be of any cell type, can originate from any type of tissue, and can be of any developmental stage, the host cell preferably is a peripheral blood lymphocyte (PBL) or a peripheral blood mononuclear cell (PBMC). More preferably, the host cell is a T cell. The T cell may be as described herein with respect to other aspects of the invention.

[0116] Also provided by an aspect of the invention is an isolated or purified population of any of the inventive cells described herein. The population of cells can be a heterogeneous population comprising the inventive cell, in addition to at least one other cell, e.g., a host cell (e.g., a T cell), which is not modified or does not comprise any of the recombinant expression vectors, or a cell other than a T cell, e.g., a B cell, a macrophage, a neutrophil, an erythrocyte, a hepatocyte, an endothelial cell, an epithelial cell, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in

which the population comprises mainly of host cells (e.g., consisting essentially of) comprising the recombinant expression vector or modified T cells. The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector or clones of a single modified T cell, such that all cells of the population comprise the recombinant expression vector or comprise the same modification. In one aspect of the invention, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein or modified T cells, as described herein.

[0117] Another aspect of the invention provides one or more mutant proteins encoded by any of the inventive nucleic acids or recombinant expression vectors described herein. The one or more mutant proteins may be as described herein with respect to other aspects of the invention. For example, the one or more mutant proteins encoded by the nucleic acid may comprise one or more of truncated SIT1, truncated LAX1 and truncated TRAT1. In another aspect of the invention, the one or more mutant proteins encoded by the nucleic acid may comprise one or more of: (i) a fusion protein comprising (a) a SIT1 extracellular domain, (b) a SIT1 transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s); (ii) a fusion protein comprising (a) a LAX1 extracellular domain, (b) a LAX1 transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s); and (iii) a fusion protein comprising (a) a TRAT1 extracellular domain, (b) a TRAT1 transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s).

[0118] The inventive mutant proteins, nucleic acids, recombinant expression vectors, and host cells (including populations thereof), can be isolated and/or purified. The term “isolated” as used herein means having been removed from its natural environment. The term “purified” as used herein means having been increased in purity, wherein “purity” is a relative term, and not to be necessarily construed as absolute purity. For example, the purity can be at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or can be about 100%.

[0119] The inventive modified T cells, mutant proteins, nucleic acids, recombinant expression vectors, host cells, and populations of cells, all of which are collectively referred to as “inventive materials” hereinafter, can be formulated into a composition, such as a pharmaceutical composition. In this regard, an aspect of the invention provides a pharmaceutical composition comprising any of the modified T cells, mutant proteins, nucleic

acids, recombinant expression vectors, host cells, and populations of cells, described herein, and a pharmaceutically acceptable carrier. The inventive pharmaceutical compositions containing any of the inventive materials can comprise more than one inventive material, e.g., a mutant protein and a nucleic acid, or two or more different mutant proteins. Alternatively, the pharmaceutical composition can comprise an inventive material in combination with another pharmaceutically active agent(s) or drug(s), such as a chemotherapeutic agent, e.g., asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, vincristine, etc.

[0120] Preferably, the carrier is a pharmaceutically acceptable carrier. With respect to pharmaceutical compositions, the carrier can be any of those conventionally used for the particular inventive material under consideration. Methods for preparing administrable compositions are known or apparent to those skilled in the art and are described in more detail in, for example, *Remington: The Science and Practice of Pharmacy*, 23rd Ed., Academic Press (2020). It is preferred that the pharmaceutically acceptable carrier be one which has no detrimental side effects or toxicity under the conditions of use.

[0121] The choice of carrier will be determined in part by the particular inventive material, as well as by the particular method used to administer the inventive material. Accordingly, there are a variety of suitable formulations of the pharmaceutical composition of the invention. Suitable formulations may include any of those for parenteral, subcutaneous, intravenous, intramuscular, intraarterial, intrathecal, intratumoral, or interperitoneal administration. More than one route can be used to administer the inventive TCR materials, and in certain instances, a particular route can provide a more immediate and more effective response than another route.

[0122] Preferably, the inventive material is administered by injection, e.g., intravenously. When the inventive material is a cell (e.g., modified T cell), the pharmaceutically acceptable carrier for the cells for injection may include any isotonic carrier such as, for example, normal saline (about 0.90% w/v of NaCl in water, about 300 mOsm/L NaCl in water, or about 9.0 g NaCl per liter of water), NORMOSOL R electrolyte solution (Abbott, Chicago, IL), PLASMA-LYTE A (Baxter, Deerfield, IL), about 5% dextrose in water, or Ringer's lactate. In an aspect, the pharmaceutically acceptable carrier is supplemented with human serum albumin.

[0123] The amount or dose (e.g., numbers of cells when the inventive material is one or more cells) of the inventive material administered should be sufficient to effect, e.g., a

therapeutic or prophylactic response, in the mammal over a reasonable time frame. For example, the dose of the inventive material should be sufficient to enhance an antigen-specific immune response, or treat or prevent a condition in a period of from about 2 hours or longer, e.g., 12 to 24 or more hours, from the time of administration. In certain aspects, the time period could be even longer. The dose will be determined by the efficacy of the particular inventive material and the condition of the mammal (e.g., human), as well as the body weight of the mammal (e.g., human) to be treated.

[0124] Many assays for determining an administered dose are known in the art. For example, an assay, which comprises comparing the extent to which target cells are lysed or IFN- γ is secreted by the modified T cells of the invention upon administration of a given dose of such modified T cells to a mammal among a set of mammals of which each is given a different dose of the modified T cells, could be used to determine a starting dose to be administered to a mammal. The extent to which target cells are lysed or IFN- γ is secreted upon administration of a certain dose can be assayed by methods known in the art.

[0125] The dose of the inventive material also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular inventive material. Typically, the attending physician will decide the dosage of the inventive material with which to treat each individual patient, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, inventive material to be administered, route of administration, and the severity of the cancer being treated. In an aspect in which the inventive material is a population of modified T cells, the number of modified T cells administered per infusion may vary, e.g., from about 1×10^6 to about 1×10^{12} cells or more. In certain aspects, fewer than 1×10^6 cells may be administered.

Methods of Treating or Preventing a Condition in a Mammal and Methods of Enhancing an Antigen-Specific Immune Response in a Mammal

[0126] It is contemplated that the inventive modified T cells, mutant proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, and pharmaceutical compositions can be used in methods of treating or preventing a condition in a mammal. In this regard, an aspect of the invention provides a method of treating or preventing a condition in a mammal, comprising administering to the mammal any of the modified T cells, populations of cells, nucleic acids, recombinant expression vectors, mutant proteins, host cells, or pharmaceutical compositions described herein, in an amount effective to treat or

prevent the condition in the mammal, wherein the condition is cancer, an infectious disease, or an autoimmune disease.

[0127] The terms “treat,” and “prevent” as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment or prevention. Rather, there are varying degrees of treatment or prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive methods can provide any amount of any level of treatment or prevention of the condition in a mammal. Furthermore, the treatment or prevention provided by the inventive method can include treatment or prevention of one or more conditions or symptoms of the condition being treated or prevented. For example, treatment or prevention can include promoting the regression of a tumor. Also, for purposes herein, “prevention” can encompass delaying the onset of the condition, or a symptom thereof. Alternatively or additionally, “prevention” may encompass preventing or delaying the recurrence of the condition, or a symptom thereof.

[0128] In an aspect of the invention, the condition is cancer. The cancer can be any cancer, including any of leukemia (e.g., B cell leukemia), sarcomas (e.g., synovial sarcoma, osteogenic sarcoma, leiomyosarcoma uteri, and alveolar rhabdomyosarcoma), lymphomas (e.g., Hodgkin lymphoma and non-Hodgkin lymphoma), hepatocellular carcinoma, glioma, head-neck cancer, acute lymphocytic cancer, acute myeloid leukemia, bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer (e.g., colon carcinoma), esophageal cancer, uterine cervical cancer, gastrointestinal carcinoid tumor, hypopharynx cancer, larynx cancer, liver cancer, lung cancer, malignant mesothelioma, melanoma, multiple myeloma, nasopharynx cancer, oropharynx, ovarian cancer, pancreatic cancer, penis, peritoneum, rectum, omentum, and mesentery cancer, pancreas, pharynx cancer, prostate cancer, rectal cancer, renal cancer, small intestine cancer, soft tissue cancer, stomach cancer, testicular cancer, thyroid cancer, ureter cancer, vagina, and urinary bladder cancer.

[0129] In an aspect of the invention, the condition is an infectious disease. The term “infectious disease” as used herein refers to any disease that results from infection with an agent. Such agents may include, for example, bacteria, viruses, fungi, and parasites, as described herein.

[0130] In an aspect of the invention, the infectious disease is a viral disease. The viral disease may affect any part of the body. The viral disease may be caused by any of the viruses described herein with respect to the viral antigen. In an aspect of the invention, the viral disease is selected from the group consisting of influenza, pneumonia, herpes, hepatitis, hepatitis A, hepatitis B, hepatitis C, chronic fatigue syndrome, sudden acute respiratory syndrome (SARS), COVID-19, gastroenteritis, enteritis, carditis, encephalitis, bronchiolitis, respiratory papillomatosis, meningitis, mononucleosis, and a pulmonary viral disease (e.g., pneumonia).

[0131] In an aspect of the invention, the infectious disease is a bacterial disease. The bacterial disease may affect any part of the body. The bacterial disease may be caused by any of the bacteria described herein with respect to the bacterial antigen. In an aspect of the invention, the bacterial disease is meningitis, tetanus, tuberculosis, gonorrhea, chlamydia, cholera, leprosy, tuberculosis, plague, syphilis, typhus, diphtheria, typhoid, dysentery, pneumonia, anthrax, listeriosis, and gastroenteritis.

[0132] In an aspect of the invention, the infectious disease is a fungal disease. The fungal disease may affect any part of the body. The fungal disease may be caused by any of the fungi described herein with respect to the fungal antigen. In an aspect of the invention, the fungal disease is selected from the group consisting of cryptococcosis, aspergillosis, coccidioidomycosis (valley fever), histoplasmosis, blastomycosis, and pneumocystis pneumonia.

[0133] In an aspect of the invention, the infectious disease is a parasitic disease. The parasitic disease may affect any part of the body. The parasitic disease may be caused by any of the parasites described herein with respect to the parasitic antigen. In an aspect of the invention, the parasitic disease is selected from the group consisting of trichomoniasis, giardiasis, cryptosporidiosis, toxoplasmosis, and malaria.

[0134] In an aspect of the invention, the condition is an autoimmune disease. Examples of autoimmune disease include, but are not limited to, rheumatoid arthritis, systemic lupus erythematosus (lupus), inflammatory bowel disease (IBD), multiple sclerosis (MS), type 1 diabetes mellitus, Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, and psoriasis.

[0135] Another aspect of the invention provides a method of enhancing an antigen-specific immune response in a mammal, the method comprising administering to the mammal any of the inventive modified T cells, population of cells, nucleic acids, recombinant

expression vectors, mutant proteins, host cells, or pharmaceutical compositions described herein, in an amount effective to enhance the antigen-specific immune response in the mammal.

[0136] An antigen-specific immune response is enhanced in accordance with the invention if the immune response to a given antigen is greater, quantitatively or qualitatively, after administration of any of the inventive materials as compared to the immune response in the absence of the administration of the inventive materials. A quantitative increase in an immune response encompasses an increase in the magnitude or degree of the response. The magnitude or degree of an immune response can be measured on the basis of any number of known parameters, such as a reduction in the size of a primary tumor or tumor metastases, an increase in the level of antigen-specific cytokine production (cytokine concentration), an increase in the number of lymphocytes activated (e.g., proliferation of antigen-specific lymphocytes) or recruited to a tumor or site of infection, and/or an increase in the production of antigen-specific antibodies (antibody concentration), etc. A qualitative increase in an immune response encompasses any change in the nature of the immune response that renders it more effective at combating a given antigen or disease. Other examples of qualitative increases in an immune response include a shift towards effector-memory or memory-type T cells providing extended efficacy or a reduction in the percentage of “exhausted” antigen-specific T cells that express markers of inactivity and are functionally compromised. Qualitative and quantitative enhancements in an immune response can occur simultaneously, and are not mutually exclusive.

Methods of Making the Cells

[0137] The inventive cells and populations of cells described herein may be made in any of a variety of different ways including, but not limited to, the methods described herein.

[0138] An aspect of the invention provides an *in vitro* method of making a modified T cell. The method may comprise introducing, into a T cell, a nucleic acid comprising a nucleotide sequence encoding one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SITI*, *LAXI* and *TRATI*. The nucleic acid comprising a nucleotide sequence encoding one or more mutant proteins may be as described herein with respect to other aspects of the invention.

[0139] In another aspect of the invention, an *in vitro* method of making a modified T cell may comprise introducing, into a pluripotent stem cell or multipotent stem cell, a nucleotide

sequence encoding one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1*.

[0140] Pluripotent stem cells have the capacity to give rise to any of the three germ layers: endoderm, mesoderm, and ectoderm. Pluripotent stem cells may comprise, for example, stem cells, e.g., embryonic stem cells, nuclear transfer derived embryonic stem cells, induced pluripotent stem cells (iPSC), etc. The pluripotent stem cells may have a stem cell phenotype including (i) the ability to self-renew and (ii) pluripotency. For example, the pluripotent stem cells, e.g., iPSCs, may be morphologically indistinguishable from embryonic stem cells (ESCs). For example, the induced pluripotent stem cells, e.g., iPSCs, may have any one or more of a round shape, large nucleolus and small volume of cytoplasm.

Alternatively or additionally, the pluripotent stem cells, e.g., iPSCs, may be any one or more of mitotically active, actively self-renewing, proliferating, and dividing. Alternatively or additionally, the pluripotent stem cells, e.g., iPSCs, may express any one or more of a variety of pluripotency-associated genes. Pluripotency-associated genes may include, but are not limited to, *Oct-3/4*, *Sox2*, *Nanog*, *GDF3*, *REX1*, *FGF4*, *ESG1*, *DPPA2*, *DPPA4*, *hTERT* and *SSEA1*.

[0141] Multipotent stem cells are cells that have the capacity to self-renew by dividing and to develop into multiple specialized cell types present in a specific tissue or organ. Multipotent stem cells produce cells of a closely related family of cells. In an aspect of the invention, the multipotent stem cells are hematopoietic stem cells.

[0142] The method may further comprise differentiating the pluripotent stem cell or multipotent stem cell with the introduced nucleotide sequence into a T cell expressing the one or more mutant proteins. In this regard, the method may comprise culturing the pluripotent stem cells or multipotent stem cells for a time and under conditions sufficient to differentiate the pluripotent or multipotent stem cells into a T cell expressing the one or more mutant proteins. Methods of differentiating pluripotent stem cells and multipotent stem cells into a variety of cell types are known in the art.

[0143] In an aspect of the invention, the method comprises introducing the nucleotide sequence encoding the one or more mutant proteins into the cell using transfection, transformation, transduction, electroporation, a transposon, or a genome editing technique. In an aspect of the invention, the genome editing technique to introduce the nucleotide sequence uses a zinc finger nuclease, transcription activator-like effector nuclease (TALENs), a CRISPR/Cas system, or engineered meganuclease.

[0144] Genome editing techniques can modify gene expression in a target cell by inserting, replacing, or removing DNA in the genome using an artificially engineered nuclease. Examples of such nucleases may include zinc finger nucleases (ZFNs) (Gomman et al., *J. Mol. Biol.*, 354(3): 507-519 (2005)), TALENs (Zhang et al., *Nature Biotechnol.*, 29: 149-153 (2011)), the CRISPR/Cas system (Cheng et al., *Cell Res.*, 23: 1163-71 (2013)), and engineered meganucleases (Riviere et al., *Gene Ther.*, 21(5): 529-32 (2014)). The nucleases create specific double-stranded breaks (DSBs) at targeted locations in the genome, and use endogenous mechanisms in the cell to repair the induced break by homologous recombination (HR) and nonhomologous end-joining (NHEJ). Such techniques may be used to suppress expression of the endogenous, wild-type subunit chains.

[0145] Another aspect of the invention provides an *in vitro* method of making a modified T cell comprising reducing one or both of expression and activity of one or more of *SIT1*, *LAX1* and *TRAT1* in the T cell using a zinc finger nuclease, TALENs, a CRISPR/Cas system, engineered meganuclease, or RNA interference, as described herein with respect to other aspects of the invention.

[0146] Another aspect of the invention provides an *in vitro* method of making a modified T cell comprising reducing one or both of expression and activity of one or more of *SIT1*, *LAX1* and *TRAT1* in a pluripotent stem cell or multipotent stem cell using a zinc finger nuclease, TALENs, a CRISPR/Cas system, engineered meganuclease, or RNA interference, as described herein with respect to other aspects of the invention. The method may further comprise differentiating the pluripotent stem cell or multipotent stem cell with the reduced one or both of expression and activity of one or more of *SIT1*, *LAX1* and *TRAT1* into a T cell with reduced one or both of expression and activity of *SIT1*, *LAX1* and *TRAT1*, as described herein with respect to other aspects of the invention.

[0147] In an aspect of the invention, the numbers of cells in the population may be rapidly expanded. Expansion of the numbers of cells (e.g., T cells) can be accomplished by any of a number of methods as are known in the art as described in, for example, U.S. Patent 8,034,334; U.S. Patent 8,383,099; U.S. Patent Application Publication No. 2012/0244133; Dudley et al., *J. Immunother.*, 26:332-42 (2003); and Riddell et al., *J. Immunol. Methods*, 128:189-201 (1990). In an aspect, expansion of the numbers of T cells is carried out by culturing the T cells with OKT3 antibody, IL-2, and feeder PBMC (e.g., irradiated allogeneic PBMC).

[0148] It is contemplated that the inventive materials described herein may be useful as an adjuvant for immune checkpoint inhibitors. In an aspect of the invention, the method of treating a condition in a mammal and the method of enhancing an antigen-specific immune response in a mammal further comprises administering an immune checkpoint inhibitor to the mammal. Examples of immune checkpoint inhibitors include, but are not limited to, PD-1 inhibitors, PD-L1 inhibitors, CTLA-4 inhibitors, and LAG-3 inhibitors.

[0149] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0150] This example demonstrates that deletion of *SIT1*, *LAX1* or *TRAT1* increases the tumoricidal activity of OTI TCR-tg CD8⁺ T cells.

[0151] Conditional (flox; f) deletion models of *SIT1*, *LAX1* and *TRAT1* were generated in mice so that the genes encoding these proteins could be inactivated by Cre recombinase. First, it was confirmed that T cell development and the phenotype of mature T cells is normal in *SIT1*^{fl/f}, *LAX1*^{fl/f} and *TRAT1*^{fl/f} mice in the absence of Cre recombinase. Next, the *dLckCre* or *ERT2Cre* transgenes were introduced into *SIT1*^{fl/f}, *LAX1*^{fl/f} and *TRAT1*^{fl/f} mice. The *dLckCre* transgene begins to express Cre in mature T cells after T cell development, whereas the *Ert2Cre* transgene can be activated by tamoxifen at any stage of development or after mature T cells are generated. Using these Cre transgenes, deletion of the *SIT1*, *LAX1* and *TRAT1* genes was induced in mature T cells, thereby avoiding any functional alterations that may occur if gene activation occurred before or during T cell development. Deletion of *SIT1*, *LAX1* and *TRAT1* was verified by screening for absence of these proteins in T cells by intracellular staining or western blot.

[0152] To provide a precise analysis of the effect of *SIT1*, *LAX1* or *TRAT1* gene deletion on TCR signaling and tumor cytotoxicity, *dLckCre* or *ERT2Cre* *SIT1*^{fl/f}, *LAX1*^{fl/f} and *TRAT1*^{fl/f} mice that express a defined transgenic MHC Class I restricted TCR, OTI, which recognizes a peptide derived from avian ovalbumin, were generated. In OTI transgenic (*OTI*-tg) mice, all mature CD8⁺ T cells express the OTI TCR and have the same antigen specificity. The tumoricidal activity of *OTI*-tg CD8⁺ T cells from *dLckCre*⁺ or *dLckCre*⁻ *SIT1*^{fl/f}, *LAX1*^{fl/f} and *TRAT1*^{fl/f} mice was evaluated (*dLckCre*⁺ versions lack expression of *SIT1*, *LAX1* or *TRAT1* whereas *dLckCre*⁻ versions express *SIT1*, *LAX1* and *TRAT1* and therefore serve as controls). B16F10 murine melanoma cells that had been engineered to

express a known high affinity (N4) or low affinity (V4) ligand for the OTI TCR, which served as pseudo-tumor specific antigens, were used as targets. Tumor cytotoxicity was measured using an xCELLigence™ impedance-based real time cell analysis (RTCA) device (Agilent Technologies, Inc., Santa Clara, CA).

[0153] Deletion of *SIT1* (Figs. 1A-1D), *LAX1* (Figs. 2A-2D) or *TRAT1* (Figs. 3A-3D) significantly increased the cytotoxic activity of *OTI-tg* CD8+ T cells against B16F10 melanoma cells expressing either the high affinity (N4) or the low affinity (V4) OTI TCR ligand. A similar enhancement of *OTI TCR-tg* CD8+ T cell cytotoxicity by deletion of *SIT1*, *LAX1*, or *TRAT1* was observed against MC38 murine colon carcinoma cells that express N4 or V4 peptide (Figs. 1A-1D, 2A-2D, and 3A-3D). Equivalent results were obtained when *SIT1*, *LAX1* and *TRAT1* were deleted by ERT2Cre (not shown).

EXAMPLE 2

[0154] This example demonstrates the effect of *SIT1*, *LAX1* or *TRAT1* wild-type, truncated or truncated-fusion proteins on OTI TCR-tg tumoricidal activity.

[0155] Next, it was investigated if “dominant negative” forms of *SIT1*, *LAX1* and *TRAT1* could be generated. These are nonfunctional mutant versions of the proteins that would compete with the endogenous wild-type versions, thereby blocking the biological activity of the wild-type proteins. It was reasoned that if dominant negative forms of *SIT1*, *LAX1* and *TRAT1* could be generated, they could be retrovirally transduced into CD8+ T cells to inhibit the activity of their wild-type counterparts. That approach, if successful, could be a more tractable (readily U.S. Food and Drug Administration-approved) method for human T cell-mediated immunotherapy compared to a CRISPR-mediated approach aimed at inactivating the *SIT1*, *LAX1* and *TRAT1* genes in T cells.

[0156] To explore this approach, retroviral constructs were generated. The retroviral constructs expressed truncated versions of murine *SIT1*, murine *LAX1* and murine *TRAT1* that retained the extracellular and transmembrane domains, but lacked almost all of the cytoplasmic domains that are assumed to perform the biological function. The truncated version of murine *SIT1* lacked the 130 C-terminal amino acid residues out of a total of 135 amino acid residues (i.e., at least 96%) of the cytoplasmic domain. The truncated version of murine *LAX1* lacked the 347 C-terminal amino acid residues out of a total of 353 amino acid residues (i.e., at least 98%) of the cytoplasmic domain. The truncated version of murine *TRAT1* lacked the 152 C-terminal amino acid residues out of a total of 159 amino acid

residues (i.e., at least 95%) of the cytoplasmic domain. As controls, retroviral constructs that express the intact, wild-type versions of murine SIT1, murine LAX1 and murine TRAT1 were also generated. The amino acid residue position numbers of the extracellular domain, transmembrane domain and cytoplasmic domain of murine SIT1, murine LAX1 and murine TRAT1 are set forth in Table 3A. The codon-optimized nucleotide sequences encoding murine SIT1, murine LAX1 and murine TRAT1 that were used to prepare the retroviral constructs are shown in Table 3B. The amino acid sequences encoded by the retroviral constructs are shown in Table 3C. In Table 3C, the transmembrane domain is shown in bold font.

TABLE 3A

	Murine SIT1 (SEQ ID NO: 14)	Murine LAX1 (SEQ ID NO: 16)	Murine TRAT1 (SEQ ID NO: 18)
Extracellular domain	Amino acid residue position numbers 1-24	Amino acid residue position numbers 1-33	Amino acid residue position numbers 1-7
Transmembrane domain	Amino acid residue position numbers 25-45	Amino acid residue position numbers 34-54	Amino acid residue position numbers 8-28
Cytoplasmic domain	Amino acid residue positions 46-180	Amino acid residue positions 55-407	Amino acid residue positions 29-187

TABLE 3B

Retroviral construct	Nucleotide sequence
Codon optimized WT murine SIT1	ATGTCCAGGGACTACAACCTGCACCACTGATGACCAGCTGGCTTGGGCGATTCCATCCATCAGCCATGCCTGGGGACTGTGGGCACCTTTTGGGTGTGGTCACTGTTCTTCTACTAATAAGCTTGGCTGCCCTGCTCTCTCAGTGGACGAGAGGTCGACGCAGGAACCAGGAGGGCCAGGGCCCTCTGAGTGGCCGCTCAGCAGAAGAAGTCCCTCTCTATGGAAATCTGCACTACTTACAGACCGGGAGGCTCAGCCAAGAACCCAGATCAGAAGAGCAGGACCCCCCAGCTCAGGAGGTCTGGCGAGAGGGGCTGAGGAGGCCATGTGCTACACATCTCTTCAACTCCGTCCTGCTCAAGGACGGATTCTCTCTGTTGGAACCCATCAAATACTGTGAAGTGTACTGGATAGTGAGCCCAAGCCACAGGCCCCCTGGGCCTGAGCCAGAGCTGTATGCATCTGTGTGTGCCAGACACGGCGAGGCCGGGCCTCCTTCCCAGATCAGGCTTATGCCAATAGCCAGCCAGCACCGAGT (SEQ ID NO: 19)
Codon optimized WT murine LAX1	ATGTACAGCACTCCAGCCCCTCCAGAGGTCACCAGAAGGAAGTCA GAACCATCCACTCGCCAAGGAACCCCTGGGATCACTGCAGGGGGA AAAAGGACAGATCATCTCCCTGGGTTTGTGCTTTTGCTCACAATA ATTCTTGTCATTATTGCAGCCTGCATCCTATGGAGCTGGAAGAAG

Retroviral construct	Nucleotide sequence
	CAGAAGAAAAGACCTGTTCCCTACTTCCAAGTAGCTCCTTCACTC ACTCTCCCACCGCCACGCCATCGGGCGAAGAACATCTACGACTTC CTGCCTCGCCAGCAGACCGAGCTCGGCCGGCACCAGCTGTCCGGC TTTTCGACAGAATCTTTACTGAGCCGAGCTAGTGACAGCCCAGAG CCCGAGGCGCCCCAGGCCAACGGCTCCCTCCAAATGCACCGCGTG TCTGTCCACACTGTAGAGTACAGTGTCAACATATATGACAACGGG ACCGTCCCACAGATGTGCAGGCATCTTGCAAGCTCCACCCACCAT GTGTGTGTGAGAAC AAGTAGGTCCAATCCTTCTATCTCTCTCGAAG GAGTCTAATGATTACGTTAATATTCCCACCGTGGAGGATACCTGT GAAACATTGACAAGGATTGAATCCACACCTGAAAACCACTTGGGA CTGCCCTCCGCTCTCCGGCTTGAATTTGCAGAAGGAGGCCATGCT GGTTGTGGGAATGCCACGGACCATGATGGCTTCTGGGCTCCGGGA CCAAAATGCAGCGACAGCCTATCAGATGGGGATGATCTTAGTCAG ACTTCCAATGACTATGTGAACATGACTGGGCTGGACCTGGAGAAC ATCCAGGAGAACCGGCCCGCTGGAGCCTTTCAGTGCTGCAGAGAT TATGAAAATGTGCCCTGGGTGGACACGAATGAGTCGCAGCTTCCG ACGTTAGAAGAAGTAGCCAGCTCTACTGTGGATCATCGAGAGCCT GTGTGGAGGACTCTAAGCAGTGTTTACCACATGGCCTTCCAGCCC AGCGCCCAGTCTGAAGACTCTGCCATGGTGCACCGGAGGAGCA GTCCAGCGAGGACTCATCAGACTATGAGACCGTACTGGTGGCAGA GCTGGAAGGTAGAGACTGGAAACAAGGCCCTGGTACCCAACACC CAAGTGATGAGGGCACCCCAGGTGACCTGGCAGGCAAGCTGTGT GAGGTGGTCTATCCTGCAGGATCTCTGGCTACAGAGACATCTGAT GAAGATGCT (SEQ ID NO: 20)
Codon optimized WT murine TRAT1	ATGTCCGGCAGCTCAGGTTGTCCCTTCTTCCTGTGGGGCCTCCTTG CCTTTTTAGGGTTGGCTCTGGTGATATCTCTAATCTTCAATATTAG CCACTATGTTGAGAAGCAACGGAGGGACGAGATCTACAGATACTC TGATGACTACATCCCCCGAGTCGATGAGTACTATGTGGAAGATGC CCCTATTTATGGCAACCTGGAGAACATCATCCCAGAGCCCCTGGA TGAAAATTGCTATGAGCAGATGAAGGGTTCGGCCACAGAGATCAG CTTCTGACCCCCAAGAAGTGGCTGCTCCTGCACAAGTCCCAGCAG AAGCCCAGATGTGCTACGCCTCGCTGGACCACAGTGTGAAAGGGA AACGTAGGCGCCCCGCGAAAGCAGAACACAAATGTTAGTGACAGA GGAAAAGACACTCAGGTATACACCATGGATGCAAATGTGTCCAG ATCAACATGGTGGAGTCCTTTCTCCAGACAACCAGGTTGTAGAA GAAAGCATTATGATGATCCTGCCCCGCTTTTTGGACTCATTAGGG CGAAGAGGGAGCCTGTCATA (SEQ ID NO: 21)

TABLE 3C

Retroviral construct	Amino acid sequence
Truncated murine SIT1	MSRDYNCTTDDQLAWGIPSISHAWGLWALLGVVTVLLL ISLAALLSQWTR (SEQ ID NO: 13)
Wild-type murine SIT1	MSRDYNCTTDDQLAWGIPSISHAWGLWALLGVVTVLLL ISLAALLSQWTRGRRRNQEGQGPLSGRSAEEVPLYGNLH

Retroviral construct	Amino acid sequence
	YLQTGRLSQEPRSEEQDPSSGGLARGAEEAMCYTSLQLR PAQGRIPSSGNPIKYCEVVLDSEPKPQAPGPEPELYASVCA QTRRGRASFPDQAYANSQPAPS (SEQ ID NO: 14)
Truncated murine LAX1	MYSTPAPPEVTRRNSEPSTRQGTGLGSLQGEKGQ IIFPGFV VLLTHLVIIAACIL WSWKK (SEQ ID NO: 15)
Wild-type murine LAX1	MYSTPAPPEVTRRNSEPSTRQGTGLGSLQGEKGQ IIFPGFV VLLTHLVIIAACIL WSWKKQKKRPVPYFQVAPSLTLPPR HRAKNIYDFLPRQQTTELGRHQLSGFSTESLLSRASDSPEPE APQANGSLQMHRVSVHTVEYSVNIYDNGTVPQMCRHLA SSTHHVCVRTSRSNPSISSKESNDYVNIPTVEDTCETLTRIE STPENHLGLPSALRLEFAEGGHAGCGNATDHDGFWAPGP KCSDSLSDGDDLSTNDYVNMTGLDLENIQENRPRGAF QCCRDYENVPWVDTNESQLPTLEEVASSTVDHREPVWRT LSSVYHMAFQPSAQSEDSAMVHREEQSESDSDYETVLV AELEGRDWKQGPQTQHPSDEGTPGDLAGKLCEVVYPAG SLATETSDEDA (SEQ ID NO: 16)
Truncated murine TRAT1	MSGSSG CPFFLWGLLAFLGLALVISL IFNISHYVE (SEQ ID NO: 17)
Wild-type murine TRAT1	MSGSSG CPFFLWGLLAFLGLALVISL IFNISHYVEKQRR DEIYRYSDDYIPRVDEYYVEDAPIYGNLENIPEPLDENCY EQMKGRPQRSASDPQEVAAAPQVPAEAQMCYASLDHSV KGKRRRPRKQNTNVSDRGKDTQVYTMDANVSQINMVES FPPDNQVVEESIHDDPARLFGLIRAKREPVI (SEQ ID NO: 18)

[0157] As predicted, overexpression of wild-type SIT1, LAX1 and TRAT1 via retroviral transduction rendered *OTI-tg* CD8⁺ T cells less effective at killing B16F10 melanoma cells compared with T cells transduced with 'empty' retroviral vector (Figs. 4A-4J, 5A-5D, and 6A-6H). Expression of truncated SIT1 (Figs. 4A-4J) or TRAT1 (Fig. 6A-6H) did not improve *OTI-tg* CD8⁺ T cell cytotoxicity, whereas expression of truncated LAX1 did significantly improve *OTI-tg* CD8⁺ T cell cytotoxicity against both B16F10-N4 and B16F10-V4 melanoma cells (Fig. 5A-5D).

[0158] Fusion proteins were also generated. The fusion proteins contained the extracellular and transmembrane domains of murine SIT1 or murine TRAT1 coupled to signaling domains from murine CD3zeta or murine CD28 similar to the approach used for the design of CARs (Xu et al., *Oncotarget*, 9:13991-14004 (2018)). The amino acid sequences encoded by the retroviral constructs are shown in Table 3D. In Table 3D, the transmembrane domain is shown in bold font, the CD3zeta sequence is shown in italics, and the CD28 sequence is underlined.

TABLE 3D

Retroviral construct	Amino acid sequence
mSIT1 truncated_CD3zITAM 1	MSRDYNCTTDDQLAWGIPSISHAWGLWALLGVVTVLLL ISLAALLSQWTRRAKFSRSAETAANLQDPNQLYNELNLGRR EEYDVLEKKRARDPEMG (SEQ ID NO: 22)
mSIT1 truncated_CD28	MSRDYNCTTDDQLAWGIPSISHAWGLWALLGVVTVLLL ISLAALLSQWTRNSRRNRLLQSDYMNMTPRRPGLTRKPY QPYAPARDFAAAYRP (SEQ ID NO: 23)
LAX1 truncated_CD3zITAM 1	MYSTPAPPEVTRRNSEPSTRQGTLGSLQGEKGQ IIFFPGFV VLLTHLVIIAACIL WSWKKRAKFSRSAETAANLQDPNQLY NELNLGRREEYDVLEKKRARDPEMG (SEQ ID NO: 24)
LAX1 truncated_CD28	MYSTPAPPEVTRRNSEPSTRQGTLGSLQGEKGQ IIFFPGFV VLLTHLVIIAACIL WSWKK NSRRNRLLQSDYMNMTPRR PGLTRKPYQPYAPARDFAAAYRP (SEQ ID NO: 25)
TRAT1 truncated_CD3zITAM 1	MSGSSGCPFFLWGLLAFLGLALVISLIFNISHYVERAKFS RSAETAANLQDPNQLYNELNLGRREEYDVLEKKRARDPEMG (SEQ ID NO: 26)
TRAT1 truncated_CD28	MSGSSGCPFFLWGLLAFLGLALVISLIFNISHYVEN NSRRN RLQSDYMNMTPRRPGLTRKPYQPYAPARDFAAAYRP (SEQ ID NO: 27)

[0159] Notably, transduction of each of the fusion proteins (SIT1truncated_CD3zetaITAM1 and SIT1truncated_CD28 or TRAT1truncated_CD3zetaITAM1 and TRAT1truncated_CD28) resulted in a significant increase in B16F10 cytotoxicity compared to the vector-only, control-transduced T cells.

EXAMPLE 3

[0160] This example demonstrates that expression of each of LAX1, SIT1 and TRAT1 is upregulated in T cells after TCR stimulation by antibody or antigen.

[0161] LAX1, SIT1 and TRAT1 are each markedly upregulated in T cells after TCR stimulation by antibody (Figure 7A) or antigen (Figure 7B). This is relevant to potential immunotherapy with T cells deleted for LAX1, SIT1 or TRAT1 since it shows that T cells upregulate these inhibitory proteins to dampen/extinguish TCR signaling.

EXAMPLE 4

[0162] This example demonstrates that dLckCre *Lax1*, *Sit1* and *Trat1* flox mice do not express LAX1, SIT1, or TRAT1, respectively.

[0163] The gene targeting DNA constructs for mouse *Lax1*, *Sit1* and *Trat1* are shown in Figures 8A, 8E, and 8C, respectively. The targeted mutations are conditional, meaning that the gene is expressed but critical exons are flanked by flox sites and can be deleted by Cre recombinase resulting in inactivation of the gene. For the experiments shown herein, the dLckCre transgene was used since dLckCre acts after T cells complete their development in the thymus (Zhang et al., *J. Immunol.*, 174:6725-6731 (2005)). This was done to mimic as closely as possible the approach that will be used for human translational applications where the genes will be inactivated in mature, fully developed T cells. The Western blots confirmed that the proteins are absent in the dLckCre *Lax1*, *Sit1* or *Trat1* flox mice (Figs. 8B, 8F, and 8D).

EXAMPLE 5

[0164] This example demonstrates that deletion of *Lax1*, *Sit1* or *Trat1* in T cells administered to tumor-bearing mice enhances control of tumor growth *in vivo* and improves survival.

[0165] Figures 1A-1D, 2A-2D, and 3A-3D show that deletion of *Lax1*, *Sit1* or *Trat1* in mouse CD8⁺ T cells that express TCRs (OT1) that recognize tumor specific antigens (in this case, avian ovalbumin derived peptides) resulted in enhanced killing of tumor cells *in vitro*. Further experiments show that deletion of *Lax1* (Figures 9A-9D and 10A-10D), *Sit1* (Figures 19A-19D and 20A-20D) or *Trat1* (Figures 28A-28D and 29A-29D) in mouse OT1 CD8⁺ T cells enhanced growth control *in vivo* of B16F10 melanomas that express either high affinity (N4) or low affinity (V4) tumor specific antigens. Consistent with these findings, tumor bearing mice injected with T cells lacking *Lax1* (Figures 11A-11D), *Sit1* (Figures 21A-21B) or *Trat1* (Figures 30A-30B) survived significantly longer than mice injected with T cells that express *Lax1*, *Sit1* and *Trat1*. A Western blot signaling experiment demonstrated that deletion of *Lax1* in CD8⁺ T cells enhanced AKT activation (pAKT; Fig. 17) as shown previously with germline *Lax1* deficient mice (Zhu et al., *J. Immunol.*, 174:5612-5619 (2005)). Figure 18 shows that the dLckCre transgene used to delete *Sit1* (and, by example, should also be true for *Lax1* and *Trat1*) yields efficient deletion of *Sit1* in mature CD4⁺ and CD8⁺ T cells after T cell development in the thymus.

EXAMPLE 6

[0166] This example demonstrates that expression of tyrosine to phenylalanine mutants of LAX1 or SIT1 in mouse CD8⁺ T cells expressing a tumor antigen-specific TCR enhances the *in vitro* cytotoxicity of the T cells against B16F10 melanoma cells that express high or low affinity target antigens.

[0167] A potential alternative to gene disruption for adoptive T cell transfer treatment of tumors is retroviral expression of a non-functional or 'dominant negative' form of a protein that might interfere with the function of the 'wild-type' version of the protein by competition, thus avoiding the necessity of deleting the gene of interest. This approach was tested by generating a set of mutant mouse Lax1 (Figure 12A), Sit1 (Figure 22A), or Tratl (Fig. 34) proteins that had phenylalanine (F) substituted for specific tyrosine (Y) residues. The mutant (Y/F) versions of Lax1, Sit1, or Tratl are depicted in Figure 12A, Figure 22A, and Figure 34, respectively. Representative retroviral transduction experiments expressed these proteins in mouse OT1 CD8⁺ T cells (Figures 12B and 22B, respectively). Figures 13A-13B show that retroviral transduction of one of the Lax1 Y/F mutants (LAX1 8YF) enhanced OT1 T cell-mediated killing of melanoma cells expressing either high affinity (N4) or low affinity (V4) tumor specific antigens even compared to *Lax1* deficient T cells. Figures 23A-23F show that several Sit1 Y/F mutants (SIT1 6YF, SIT1 73F + 153F and SIT1 153F), when retrovirally transduced into mouse OT1 CD8⁺ T cells, enhanced the *in vitro* cytotoxic activity of OT1 CD8⁺ T cells relative to control (empty vector) transduced OT1 CD8⁺ T cells.

EXAMPLE 7

[0168] This example demonstrates deletion of LAX1, SIT1 or TRAT1 in human CD8⁺ T cells expressing a tumor antigen-specific TCR enhances cytokine production by the T cells in response to high or low affinity tumor antigens and enhances killing of melanoma cells expressing high or low affinity tumor antigens.

[0169] The enhanced tumor cytotoxicity observed with mouse CD8⁺ T cells rendered deficient in *LAX1*, *SIT1* or *TRAT1* was confirmed *in vitro* with human CD8⁺ T cells. Human donor PBMCs were electroporated with Cas9 protein and a set of 2-3 sgRNAs directed against *LAX1* (Figures 14A-14B), *SIT1* (Figures 24A-24B) or *TRAT1* (Figures 31A-31B) to extinguish expression of these genes by CRISPR/Cas9 mutagenesis. The sequences of the sgRNAs are provided in Table 4, below). Next, the T cells were retrovirally transduced with

retrovirus encoding human/mouse chimeric TCR (1G4) that recognizes a human cancer-testis tumor antigen, NY-ESO1 (Tsimberidou et al., *Hum. Vaccin. Immunother.*, 19:2290356 (2023)). 1G4+ T cells lacking LAX1 (Figure 15A-15H), SIT1 (Figures 25A-25H) or TRAT1 (Figure 32A-32F) were significantly superior to control LAX1, SIT1, TRAT1 expressing 1G4+ T cells at killing SK-MEL human melanoma cells that expressed NY-ESO1 peptide 9V. LAX1, SIT1 or TRAT1 deficient T cells were also superior to control T cells at killing SK-MEL cells that expressed single amino acid variant peptides of NY-ESO1 that bound to 1G4 with lower affinity than 9V ranging from high-4A-5Y-8S-low (Figures 15A-15H, 25A-25H, and 32A-32F). Moreover, LAX1 deficient T cells expressed more of the inflammatory cytokine TNF α (Figure 16) than control LAX1 replete T cells. SIT1 deficient T cells expressed more IL-2 and IFN γ (Figures 26A-26B) and the cytotoxic proteins perforin and granzyme B (Figures 27A-27B) than control SIT1 replete T cells. TRAT1 deficient T cells expressed more IFN γ and TNF α (Figures 33A-33C) than control TRAT1 replete T cells.

TABLE 4

SEQ ID NO: 28 Hs.Cas9.TRAT1.1.AA	mC*mU*mU*rArArCrGrCrUrGrUrGrArUrCrArArGrUrGrGr UrUrUrUrArGrArGrCrUrArGrArArArUrArGrCrArArGrUrUr ArArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArC rUrUrGrArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUr GrCmU*mU*mU*rU
SEQ ID NO: 29 Hs.Cas9.TRAT1.1.AC	mA*mU*mU*rGrArArGrArCrArCrArCrArArUrUrUrArGr UrUrUrUrArGrArGrCrUrArGrArArArUrArGrCrArArGrUrUr ArArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArC rUrUrGrArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUr GrCmU*mU*mU*rU
SEQ ID NO: 30 Hs.Cas9.SIT1.1.AA	mC*mU*mG*rCrArCrGrGrArUrCrUrArCrUrCrGrCrArCrGrU rUrUrUrArGrArGrCrUrArGrArArArUrArGrCrArArGrUrUrAr ArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArCrU rUrGrArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGr CmU*mU*mU*rU
SEQ ID NO: 31 Hs.Cas9.SIT1.1.AB	mC*mU*mC*rCrArArGrArGrUrUrGrGrArUrCrCrUrGrCrGrU rUrUrUrArGrArGrCrUrArGrArArArUrArGrCrArArGrUrUrAr

	ArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArCrUrUrGrArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCmU*mU*mU*rU
SEQ ID NO: 32 Hs.Cas9.SIT1.1.AC	mA*mA*mG*rCrArCrArCrUrGrCrUrCrUrGrArGrCrCrGrGrUrUrUrArGrArGrCrUrArGrArArArUrArGrCrArArGrUrUrArArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArCrUrUrGrArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCmU*mU*mU*rU
SEQ ID NO: 33 Hs.Cas9.LAX1.1.AA	mA*mG*mG*rGrCrArUrGrArCrGrGrUrArArCrUrCrGrGrGrUrUrUrUrArGrArGrCrUrArGrArArArUrArGrCrArArGrUrUrArArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArCrUrUrGrArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCmU*mU*mU*rU
SEQ ID NO: 34 Hs.Cas9.LAX1.1.AB	mC*mA*mU*rGrCrCrArCrArGrArGrUrArCrGrCrGrGrUrGrUrUrUrUrArGrArGrCrUrArGrArArArUrArGrCrArArGrUrUrArArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArCrUrUrGrArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCmU*mU*mU*rU
SEQ ID NO: 35 Hs.Cas9.LAX1.1.AC	mA*mA*mC*rCrGrCrArArCrGrArCrCrArGrGrArGrGrArGrUrUrUrUrArGrArGrCrUrArGrArArArUrArGrCrArArGrUrUrArArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArCrUrUrGrArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCmU*mU*mU*rU
Legend for Table 4: mA is 2'O-methyladenosine. mG is 2'O-methylguanosine. mC is 2'O-methylcytosine. mU is 2'O-methyluridine. * (asterisk) is a phosphorothioate bond. “r” indicates that the following base is an RNA base.	

[0170] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were

individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0171] The use of the terms “a” and “an” and “the” and “at least one” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term “at least one” followed by a list of one or more items (for example, “at least one of A and B”) is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0172] Preferred aspects of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred aspects may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. A T cell expressing an antigen-specific receptor, wherein the T cell has been modified to reduce one or both of expression and activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1*.
2. The T cell according to claim 1, wherein the antigen-specific receptor is an endogenous T cell receptor (TCR), an exogenous TCR, a chimeric antigen receptor (CAR), a bispecific engager TCR fusion protein, T cell antigen coupler (TAC), a T cell receptor fusion construct, or a synthetic TCR and antigen receptor (STAR).
3. The T cell according to claim 1 or 2, wherein the T cell is derived from an induced pluripotent stem cell (iPSC), embryonic stem cell or hematopoietic stem cell.
4. The cell according to any one of claims 1-3, wherein the T cell is a natural killer T (NKT) cell, a mucosal-associated invariant T (MAIT) cell, an invariant natural killer T (iNKT) cell, a gamma delta ($\gamma\delta$) T cell, an alpha beta ($\alpha\beta$) T cell, a tumor infiltrating lymphocyte (TIL), or a regulatory T cell (Treg).
5. The T cell according to any one of claims 1-4, wherein the T cell is modified to express one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1*.
6. The T cell according to claim 5, wherein the one or more mutant proteins comprise one or more of truncated SIT1, truncated LAX1 and truncated TRAT1.
7. The T cell according to claim 5, wherein the one or more mutant proteins comprise one or more of:
 - (i) a fusion protein comprising (a) a SIT1 extracellular domain, (b) a SIT1 transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s);

(ii) a fusion protein comprising (a) a LAX1 extracellular domain, (b) a LAX1 transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s); and

(iii) a fusion protein comprising (a) a TRAT1 extracellular domain, (b) a TRAT1 transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s).

8. The T cell of any one of claims 1-7, comprising an inactivating mutation of (i) gene encoding or (ii) a promoter sequence regulating expression of a gene encoding one or more of *SIT1*, *LAX1* and *TRAT1*.

9. The T cell of claim 5, wherein the one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1* comprise one or more of:

(i) a human *SIT1* amino acid sequence, wherein the tyrosine at one or both of positions 90 and 168, as defined by reference to SEQ ID NO: 2 is, independently, substituted with another amino acid;

(ii) a human *TRAT1* amino acid sequence, wherein the tyrosine at one or more of positions 43, 45, 79, and 110, as defined by reference to SEQ ID NO: 12 is, independently, substituted with another amino acid; and

(iii) a human *LAX1* amino acid sequence, wherein the tyrosine at five or more of positions 71, 93, 150, 155, 193, 268, 294, and 373, as defined by reference to SEQ ID NO: 8 is, independently, substituted with another amino acid.

10. The T cell of claim 5, wherein the one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1* comprise one or more of:

(i) a human *SIT1* amino acid sequence, wherein the tyrosine at one or both of positions 90 and 168, as defined by reference to SEQ ID NO: 2 is, independently, substituted with phenylalanine;

(ii) a human *TRAT1* amino acid sequence, wherein the tyrosine at one or more of positions 43, 45, 79, and 110, as defined by reference to SEQ ID NO: 12 is, independently, substituted with phenylalanine; and

(iii) a human LAX1 amino acid sequence, wherein the tyrosine at five or more of positions 71, 93, 150, 155, 193, 268, 294, and 373, as defined by reference to SEQ ID NO: 8 is, independently, substituted with phenylalanine.

11. The T cell of any one of claims 1-11, comprising a nucleic acid comprising a nucleotide sequence encoding an RNA interference (RNAi) agent that reduces one or both of expression and activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1*.

12. The T cell according to any one of claims 1-11, wherein the antigen-specific receptor has antigenic specificity for a cancer antigen, an autoimmune disease related self-antigen or an infectious disease antigen.

13. A population of cells comprising at least one T cell according to any one of claims 1-12.

14. A nucleic acid comprising a nucleotide sequence encoding one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1*.

15. The nucleic acid according to claim 14, wherein the one or more mutant proteins comprise one or more of truncated SIT1, truncated LAX1 and truncated TRAT1.

16. The nucleic acid according to claim 14, wherein the one or more mutant proteins comprise one or more of:

(i) a fusion protein comprising (a) a SIT1 extracellular domain, (b) a SIT1 transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s);

(ii) a fusion protein comprising (a) a LAX1 extracellular domain, (b) a LAX1 transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s); and

(iii) a fusion protein comprising (a) a TRAT1 extracellular domain, (b) a TRAT1 transmembrane domain, and (c) one or more TCR and/or T cell co-receptor intracellular signaling domain(s).

17. The nucleic acid of claim 14, wherein the one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1* comprise one or more of:

(i) a human *SIT1* amino acid sequence, wherein the tyrosine at one or both of positions 90 and 168, as defined by reference to SEQ ID NO: 2 is, independently, substituted with another amino acid;

(ii) a human *TRAT1* amino acid sequence, wherein the tyrosine at one or more of positions 43, 45, 79, and 110, as defined by reference to SEQ ID NO: 12 is, independently, substituted with another amino acid; and

(iii) a human *LAX1* amino acid sequence, wherein the tyrosine at five or more of positions 71, 93, 150, 155, 193, 268, 294, and 373, as defined by reference to SEQ ID NO: 8 is, independently, substituted with another amino acid.

18. The nucleic acid of claim 14, wherein the one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1* comprise one or more of:

(i) a human *SIT1* amino acid sequence, wherein the tyrosine at one or both of positions 90 and 168, as defined by reference to SEQ ID NO: 2 is, independently, substituted with phenylalanine;

(ii) a human *TRAT1* amino acid sequence, wherein the tyrosine at one or more of positions 43, 45, 79, and 110, as defined by reference to SEQ ID NO: 12 is, independently, substituted with phenylalanine; and

(iii) a human *LAX1* amino acid sequence, wherein the tyrosine at five or more of positions 71, 93, 150, 155, 193, 268, 294, and 373, as defined by reference to SEQ ID NO: 8 is, independently, substituted with phenylalanine.

19. A recombinant expression vector comprising the nucleic acid according to any one of claims 14-18.

20. A mutant protein encoded by the nucleic acid of any one of claims 14-18 or the recombinant expression vector of claim 19.

21. A host cell comprising the recombinant expression vector of claim 19.

22. A population of cells comprising at least one host cell of claim 21.
23. A pharmaceutical composition comprising (i) a pharmaceutically acceptable carrier and (ii) the T cell according to any one of claims 1-12, the population of cells according to claim 13 or 22, the nucleic acid of any one of claims 14-18, the recombinant expression vector of claim 19, the mutant protein of claim 20, or the host cell of claim 21.
24. The T cell according to any one of claims 1-12, the population of cells according to claim 13 or 22, the nucleic acid of any one of claims 14-18, the recombinant expression vector of claim 19, the mutant protein of claim 20, the host cell of claim 21, or the pharmaceutical composition according to claim 23, for use in the treatment of a condition in a mammal, wherein the condition is cancer, an infectious disease, or an autoimmune disease.
25. An *in vitro* method of making a modified T cell, the method comprising reducing one or both of expression and activity of one or more of *SIT1*, *LAX1* and *TRAT1* in the T cell using a zinc finger nuclease, transcription activator-like effector nuclease (TALENs), a CRISPR/Cas system, engineered meganuclease, or RNA interference.
26. An *in vitro* method of making a modified T cell, the method comprising introducing, into a T cell, a nucleic acid comprising a nucleotide sequence encoding one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1*.
27. An *in vitro* method of making a modified T cell, the method comprising:
introducing, into a pluripotent stem cell or multipotent stem cell, a nucleotide sequence encoding one or more mutant proteins that reduce the activity of polypeptide(s) encoded by one or more of *SIT1*, *LAX1* and *TRAT1*, and
differentiating the pluripotent stem cell or multipotent stem cell with the introduced nucleotide sequence into a T cell expressing the one or more mutant proteins.
28. The method of claim 26 or 27, wherein the nucleotide sequence is introduced into the cell using transfection, transformation, transduction, electroporation, a transposon, or a genome editing technique.

29. The method of claim 28, wherein the genome editing technique uses a zinc finger nuclease, transcription activator-like effector nuclease (TALENs), a CRISPR/Cas system, or engineered meganuclease.

30. An *in vitro* method of making a modified T cell, the method comprising:

reducing one or both of expression and activity of one or more of *SIT1*, *LAX1* and *TRAT1* in a pluripotent stem cell, a multipotent stem cell, or a lymphoid cell derived from a pluripotent stem cell or multipotent stem cell, using zinc finger nuclease, transcription activator-like effector nuclease (TALENs), a CRISPR/Cas system, engineered meganuclease, or RNA interference, and

differentiating the pluripotent stem cell or multipotent stem cell with the reduced one or both of expression and activity of one or more of *SIT1*, *LAX1* and *TRAT1* into a T cell with reduced one or both of expression and activity of *SIT1*, *LAX1* and *TRAT1*.

31. The T cell according to any one of claims 1-12, the population of cells according to claim 13 or 22, the nucleic acid of any one of claims 14-18, the recombinant expression vector of claim 19, the mutant protein of claim 20, the host cell of claim 21, or the pharmaceutical composition according to claim 23, for use in the enhancement of an antigen-specific immune response in a mammal.

32. The T cell, population of cells, nucleic acid, recombinant expression vector, mutant protein, host cell, or pharmaceutical composition of claim 24 or 31, further comprising an immune checkpoint inhibitor, for the use of claim 24 or 31.