Background

[0004] Various strategies are available for stimulating T cell populations *in vitro*, including for expanding antigen-specific T cells *in vitro* for use in adoptive cellular immunotherapy or cancer therapy in which infusions of such T cells have been shown to have anti-tumor reactivity in a tumor-bearing host or for use to treat viral infections. Improved strategies are needed for expanding cell populations *in vitro*, including for research, diagnostic and therapeutic purposes. Provided are reagents, methods, articles of manufacture and kits that meet such needs.

Summary

[0004a] In one aspect, the present invention provides a method for culturing T cells, the method comprising:

(a) incubating a composition comprising T cells in the presence of a receptor-binding agent, wherein the receptor-binding agent i) is reversibly bound to a reagent comprising a plurality of binding sites capable of reversibly binding to the receptor-binding agent and ii) specifically binds to a molecule on the surface of the T cells in a manner that induces or modulates a signal in T cells in the composition; and

(b) within 5 days after initiation of said incubation, disrupting the reversible binding between the receptor-binding agent and the reagent, thereby generating cultured T cells.

[0004b] In another aspect, the present invention provides a method for culturing T cells, the method comprising:

(a) incubating a composition comprising T cells in the presence of:

i) a first receptor-binding agent that specifically binds to a first molecule expressed on the surface of the T cells in a manner that induces or modulates a TCR/CD3 complex-associated signal in the T cell; and

ii) a second receptor-binding agent, wherein the second receptor-binding agent i) is reversibly bound to a reagent comprising a plurality of binding sites capable of reversibly binding to the second receptor-binding agent and ii) specifically binds to a second molecule on the surface of the T cells in a manner to induce or modulate a second signal in the T cells; and

(b) within 5 days after initiation of said incubation, disrupting the reversible binding between the second receptor-binding agent and the reagent, thereby generating cultured T cells.

[0005] Provided herein are methods for incubating cells and compositions thereof, such as for culturing such cells, using reversible reagents for inducing or modulating a signal in a target cell. In some examples, the cells are T cells.

Disclosed is that the method involves incubating a composition containing T cells in the presence of an agent, such as a receptor-binding agent, *e.g.*, a stimulatory agent. The receptor-binding agent (*e.g.*, stimulatory agent) may be reversibly bound to a reagent containing a plurality of binding sites capable of reversibly binding to the receptor-binding agent. Disclosed is that the receptor-binding agent is capable of specifically binding to a molecule on the surface of the cells, *e.g.*, T cells, such as in a manner that induces or modulates a signal in the cells, *e.g.*, the T cells in the composition. In some examples, the methods involve features, such as particular steps, selection of particular agents and/or selection of particular reagents, which allow the control or adjustment of the type or strength or duration of the signal received or modulated via the reagent, and/or of properties of the output composition or cell population(s) ultimately generated by the methods. In some examples, such features are possible due to advantageous properties of the agents and reagents, such as the reversibility of binding of the individual components of the agents or reagents, and thus, the reversibility of the binding of the multimerized agents and the cells. Such properties of the reagents can be exploited to achieve control in a number of ways. For example, in some examples, the methods, via reversibility of binding, include exerting temporal control of the signal, controlling the duration of the period in which the cells are in contact with the multimerized agents, and/or the duration of the signaling induced thereby.

[0006] In some examples, the methods involve control, *e.g.*, precise control, of the length of the time period under which such agents are bound to cells. For example, this may be

done by actively reversing such binding at a particular timepoint, and in some cases while still maintaining the cells for an additional time period other culture conditions, such as incubation at a physiological temperature and/or with various nutrients. Thus, as opposed to other methods which simply involve the termination of all or substantially all signals received by the cells, the provided methods in some examples allow the *specific* termination or disruption of signals delivered by particular reagents.

[0008] Likewise, in some examples, the reversibility allows the reagents to be modular in nature, permitting the substitution of one or more components thereof without engineering or new reagents, *e.g.*, by simply reversing binding and combining with additional agents or reagents, under conditions where reversible binding is induced. For example, by being able to reversibly bind various stimulatory agents to the same multimerization reagent, either at the same time or at different times, the user of the provided methods and compositions may adjust the nature of the particular signal being delivered, *e.g.*, by substituting one or more stimulatory agent for another one or more stimulatory agent, such as to induce a stronger or weaker or qualitatively different signal, depending on the desired outcome.

[0009] In some examples, temporal control and modularity are used in combination, *e.g.*, by incubating cells in the presence of one agent for a certain period of time, inducing reversal of binding by disruption, followed by incubation in the presence of other or more different agents or reagents. For example, in one examples, T cells are initially stimulated with a reagent to deliver a particular strength or quality of signal, and after a certain period of time, such signal is disrupted and a qualitatively or quantitatively different (*e.g.*, stronger or weaker or activating different signaling pathways or known to be important for different differentiation pathways) signal is substituted. In some examples, such control provides advantages, for example, allowing the user to maximize desired outcomes (*e.g.*, expansion or persistence) while avoiding undesirable outcomes such as exhaustion or anergy.

[0010] In some examples, temporal control is achieved by disrupting the reversible binding of the reagents or agents, such as by the addition of a substance. For example, in some examples, within a period of time, *e.g.*, within 5 days after initiation of the incubation, and/or within a certain percentage of the total length of the incubation, such as within 1/5, ¼, 1/3, or 1/2 of the time, the reversible binding between the receptor-binding agent and the reagent is disrupted. Thus, in some cases the method results in the generation of cultured T cells.

[0011] In some examples, the incubation is performed under conditions in which the receptor-binding agent specifically binds to the molecule, thereby inducing or modulating the signal in one or more T cells in the composition.

[0012] In some examples, the disruption of the binding between the receptor-binding agent and the reagent is effected more than 30 minutes after the initiation of the incubation. For example, disclosed is that the disruption of the binding between the receptor-binding agent and the reagent is effected between 1 hour and 4 days after initiation of the incubation, between 6 hours and 3 days after initiation of the incubation, between 12 hours and 2 days after initiation of the incubation, or between 1 day and 3 days after initiation of the incubation. In some cases, the disruption of the binding between the receptor-binding agent and the reagent is effected between about 1 hour and about 4 days after initiation of the incubation, between about 6 hours and about 3 days after initiation of the incubation, between about 12 hours and about 2 days after initiation of the incubation, or between about 1 day and about 3 days after initiation of the incubation. Disclosed is that the disruption is effected greater than or equal to about 1 hour after initiation of said incubation and within 1 day, 2 days, 3 days or 4 days after initiation of the incubation.In some examples, binding of the receptor-binding agent is capable of initiatingor does initiates a TCR/CD3 complex-associated signal in the T cells. Disclosed is that the receptor-binding agent specifically binds to a member of a TCR/CD3 complex. In some instances, the receptor-binding agent specifically binds to CD3.

[0013] In some examples, the molecule (the molecule on the surface of the T cells) is a component of the TCR/CD3 complex or is CD3. Disclosed is that the molecule is a first molecule and the receptor-binding agent is further capable of specifically binding to a second molecule on the surface of one or more of the T cells. In some cases the second molecule is capable of inducing or enhancing, dampening, or modifying a signal delivered through the first molecule in the T cells.

[0014] Disclosed is that the receptor-binding agent includes a binding partner C1. Disclosed is that the plurality of binding sites contained by the reagent includes two or more binding sites, Z1. In some instances, the two or binding sites Z1 each are capable of binding to the binding partner C1 to form the reversible bond between the receptor-binding agent and the reagent. In some examples, the disruption of the binding between the receptor-binding agent and the reagent includes introducing to the cells a composition containing a substance capable of reversing the bond between the receptor-binding agent and the reagent.

[0015] In some examples, the receptor-binding agent is a first receptor-binding agent and the incubation is further carried out in the presence of a second receptor-binding agent, which is capable of specifically binding to a second molecule on the surface of one or more of the T cells. In some examples, the binding of the second receptor-binding agent to the second molecule or does enhance, dampen, or modify a signal delivered through the first molecule in the T cells.

[0016] In some examples, the reagent contains a plurality of binding sites capable of reversibly binding to the second receptor-binding agent. In some such cases, the second receptor-binding agent is reversibly bound to the reagent. Disclosed is that the plurality of binding sites capable of reversibly binding to the first receptor-binding agent and the plurality of binding sites capable of reversibly binding to the second receptor-binding agent can be the same or can be different.

[0017] In some ects, the second receptor-binding agent includes a binding partner C1 or C2, which is capable of reversibly binding to the two or more binding sites Z1. In some such instances, the first and second receptor-binding agents are reversibly bound to the reagent via the two or more binding sites Z1. In some cases, the second receptor-binding agent contains a binding partner C2 and the reagent further contains a plurality of binding sites Z2. The plurality of binding sites Z2 may be capable of binding to the binding partner C2 to form the reversible bond between the second receptor-binding agent and the reagent. Disclosed is that C2 and C1 are the same or substantially the same, or contain the same or substantially the same moiety. In some instances, Z1 and Z2 are the same or substantially the same or contain the same or substantially the same moiety.

[0018] In some cases, the reagent is a first reagent and the incubation is carried out in the presence of at least a second reagent which is reversibly bound to the second receptor-binding agent. In some examples, the incubation is performed under conditions in which the second receptor-binding agent specifically binds to the second molecule. Disclosed is that the bind of the second receptor-binding agent to the second molecule induces or modulates a signal, *e.g.*, that enhances, dampens, or modifies a signal delivered through the first molecule in the T cells.

[0019] In some examples, said disruption includes introducing to the cells a composition that contains a substance capable of reversing the bond between the first receptor-binding agent and the reagent and/or the second receptor-binding agent and the reagent. In some examples, said disruption terminates or lessens the signal induced or modulated by the first

receptor-binding agent and terminates or lessens the signal induced or modulated by the second receptor-binding agent.

[0020] Provided herein in some examplesis a method for culturing T cells that includes incubating a composition containing T cells in the presence of a first receptor-binding agent that is capable of specifically binding to a first molecule expressed on the surface of the T cells. Disclosed is that the binding of the first receptor-binding agent to the first molecule induces or modulates a TCR/CD3 complex-associated signal in the T cell. In some such examples, the composition further is incubated in the presence of a second receptor-binding agent that is reversibly bound to a reagent containing a plurality of binding sites capable of reversibly binding to the second receptor-binding agent. Disclosed is that the second receptor-binding agent is capable of specifically binding to a second molecule on the surface of the T cells. In some cases, the binding of the second receptor-binding agent to the second molecule induces or modulates a second signal in the T cell, such as to enhance, dampen or modify a signal delivered through the first molecule. Disclosed is that within 5 days after initiation of the incubation, the reversible binding between the second receptor-binding agent and the reagent is disrupted, thereby generating cultured T cells.

[0021] In some examples, the second signal enhances, dampens or modifies a signal delivered through the first molecule in the T cells.

[0022] In some examplesof any of the methods provided herein, the incubation is performed under conditions in which the first receptor-binding agent specifically binds to the first molecule and/or the second receptor-binding agent specifically binds to the second molecule, thereby inducing or modulating one or more signals in the T cells. In some examples, said disruption is carried out greater than 30 minutes after initiation of said incubation. In some examples, said disruption is carried out between 1 hour and 4 days after initiation of said incubation, between 6 hours and 3 days after initiation of said incubation, between 12 hours and 2 days after initiation of said incubation or between 1 day and 3 days after initiation of said incubation; or said disruption is carried out between about 1 hour and about 4 days after initiation of said incubation, between about 6 hours and about 3 days after initiation of said incubation, between about 12 hours and about 2 days after initiation of said incubation or between about 1 day and about 3 days after initiation of said incubation; or said disruption is carried out greater than or equal to about 1 hour after initiation of said incubation and within 1 day, 2 days, 3 days or 4 days after initiation of said incubation.

[0023] In some examples, the second receptor-binding agent includes a binding partner C1. In some such examples, the plurality of binding sites contained by the reagent includes two or more binding sites, Z1, which each are capable of binding to the binding partner C1 to form the reversible bond between the second receptor-binding agent and the reagent.

[0024] In some examplesof any of the methods provided herein, the second signal is a signal other than a TCR/CD3 complex-associated signal; the second signal is capable of enhancing or potentiating a TCR/CD3 complex-associated signal; or the second molecule is a costimulatory molecule, accessory molecule, cytokine receptor, chemokine receptor, immune checkpoint molecule or is a member of the TNF family or the TNF receptor family. In some examples, the second molecule is selected from among CD28, CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40 and HVEM. In some examples, the second molecule is CD28.

[0025] In some examples, the second molecule is or includes an adhesion molecule or is a factor that induces cytokine production, chemokine production and/or expression of an adhesion molecule.

[0026] In some examples, the second receptor-binding agent specifically binds to a cytokine receptor selected from among IL-2R, IL-1R, IL-15R, IFN-gammaR, TNF-alphaR, IL-4R, IL-10R, Type I IFNR, IL-12R, IL-15R, IL-17R, TNFR1 and TNFR2. In some examples, the second receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among IL-2R, IL-1R, IL-15R, IFN-gammaR, TNF-alphaR, IL-4R, IL-10R, Type I IFNR, IL-12R, IL-15R, IL-17R, TNFR1 and TNFR2; and/or the second receptor-binding agent is a ligand selected from among IL-2, IL-1, IL-15, IFN-gamma, TNF-alpha, IL-4, IL-10, IL-12, IL-15, IL-17 and TNF, or is a biologically active fragment thereof. In some examples, the second receptor-binding agent specifically binds to a cytokine receptor selected from among IL-12R, IFN-gammaR, IL-4R and IL-17R; or the second receptor-binding agent is a ligand selected from among IL-2, IL-4, IL-7, IL-10, IL-15 and IL-17 or is a biologically active fragment thereof.

[0027] In some examples, the second receptor-binding agent specifically binds to a cytokine receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain). In some examples, the second receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain); and/or the second receptor-binding agent is a ligand selected from among IL-7, IL-21, IL-2, IL-4, IL-9 and IL-15, or is a biologically active fragment thereof. In

some examples, the second receptor-binding agent specifically binds to a cytokine receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain); or the second receptor-binding agent is a ligand selected from among IL-7, IL-21, IL-2, IL-4, IL-9 and IL-15, or is a biologically active fragment thereof.

[0028] In some examples, the second receptor-binding agent specifically binds to a chemokine receptor selected from among CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR9, CXCR1, CXCR3 and CXCR4. In some examples, the second receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR9, CXCR1, CXCR3 and CXCR4; or the second receptor-binding agent is a ligand selected from among CXCL9, CXCL10, CCL19, CCL21 and CCL25 or is a biologically active fragment thereof. In some examples, the second receptor-binding agent specifically binds to a chemokine receptor selected from among CXCR3, CCR7, CXCR1 and CXCR4; or the second receptor-binding agent is a ligand selected from among CXCL9, CXCL10, CCL19, CCL21 and CCL25 or is a biologically active fragment thereof.

[0029] In some examples, the adhesion molecule is selected from among CD44, CD31, CD18/CD11a (LFA-1), CD29, CD54 (ICAM-1), CD62L (L-selectin), and CD29/CD49d (VLA-4), CD106 (VCAM-1) or is a biologically active fragment thereof. In some examples, the adhesion molecule is selected from among LFA-1, L-selectin, VCAM-1 and VLA-4 or is a biologically active fragment thereof.

[0030] In some examples, the factor is a nuclear factor. In some examples, the factor is a retinoic acid receptor-related orphan receptor gamma (RORgamma) or RORalpha.

[0031] In some examples, said disruption includes introducing to the cells a composition that contains a substance capable of reversing the bond between the second receptor-binding agent and the reagent. In some examples, said disruption terminates or lessens the signal induced or modulated by the second receptor-binding agent. For example, in some examples, the second molecule is CD28 and the disruption terminates or lessens the CD28 costimulatory signal in the T cells.

[0032] In some examples, the incubation is further carried out in the presence of a third receptor-binding agent, which is capable of specifically binding to a third molecule on the surface of one or more of the T cells.

[0033] In some examplesof any of the methods provided herein, the first receptor-binding agent specifically binds to a first molecule expressed on the surface of the T cells in a

manner that induces or modulates a TCR/CD3 complex-associated signal in the T cell; the second receptor-binding agent specifically binds to the second molecule, thereby inducing or modulating a second signal in the T cells; and the third receptor-binding agent specifically binds to a third molecule on the surface of the T cells that induces or modulates a further signal in the cell.

[0034] In some examples, the first molecule is CD3.

[0035] In some examples, the second signal enhances, dampens or modifies a signal delivered through the first molecule. In some examples, the second molecule is a costimulatory molecule, accessory molecule, cytokine receptor, chemokine receptor, immune checkpoint molecule or is a member of the TNF family or the TNF receptor family. In some examples, the second molecule is selected from among CD28, CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40 and HVEM. In some examples, the second molecule is CD28.

[0036] In some examples, the third molecule is a cytokine receptor, a chemokine receptor, or is or includes an adhesion molecule or is a factor that induces cytokine production, chemokine production and/or expression of an adhesion molecule. In some examples, the third receptor-binding agent specifically binds to a cytokine receptor selected from among IL-2R, IL-1R, IL-15R, IFN-gammaR, TNF-alphaR, IL-4R, IL-10R, Type I IFNR, IL-12R, IL-15R, IL-17R, TNFR1 and TNFR2. In some examples, the third receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among IL-2R, IL-1R, IL-15R, IFN-gammaR, TNF-alphaR, IL-4R, IL-10R, Type I IFNR, IL-12R, IL-15R, IL-17R, TNFR1 and TNFR2; and/or the third receptor-binding agent is a ligand selected from among IL-2, IL-1, IL-15, IFN-gamma, TNF-alpha, IL-4, IL-10, IL-12, IL-15, IL-17 and TNF, or is a biologically active fragment thereof.

[0037] In some examples, the third receptor-binding agent specifically binds to a cytokine receptor selected from among IL-12R, IFN-gammaR, IL-4R and IL-17R; or the third receptor-binding agent is a ligand selected from among IL-2, IL-4, IL-7, IL-10, IL-15 and IL-17 or is a biologically active fragment thereof. In some examples, the third receptor-binding agent specifically binds to a cytokine receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain). In some examples, the third receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain); and/or the third receptor-binding agent is a ligand

selected from among IL-7, IL-21, IL-2, IL-4, IL-9 and IL-15, or is a biologically active fragment thereof. In some examples, the third receptor-binding agent specifically binds to a cytokine receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain); or the third receptor-binding agent is a ligand selected from among IL-7, IL-21, IL-2, IL-4, IL-9 and IL-15, or is a biologically active fragment thereof.

[0038] In some examples, the third receptor-binding agent specifically binds to a chemokine receptor selected from among CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR9, CXCR1, CXCR3 and CXCR4. In some examples,: the third receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR9, CXCR1, CXCR3 and CXCR4; or the third receptor-binding agent is a ligand selected from among CXCL9, CXCL10, CCL19, CCL21 and CCL25 or is a biologically active fragment thereof. In some examples, the third receptor-binding agent specifically binds to a chemokine receptor selected from among CXCR3, CCR7, CXCR1 and CXCR4; or the third receptor-binding agent is a ligand selected from among CXCL9, CXCL10, CCL19, CCL21 and CCL25 or is a biologically active fragment thereof.

[0039] In some examples, the adhesion molecule is selected from among CD44, CD31, CD18/CD11a (LFA-1), CD29, CD54 (ICAM-1), CD62L (L-selectin), and CD29/CD49d (VLA-4), CD106 (VCAM-1) or is a biologically active fragment thereof. In some examples, the adhesion molecule is selected from among LFA-1, L-selectin, VCAM-1 and VLA-4 or is a biologically active fragment thereof.

[0040] In some examples, the factor is a nuclear factor. In some examples, the factor is a retinoic acid receptor-related orphan receptor gamma (RORgamma) or RORalpha.

[0041] In some examples, the third receptor-binding agent is reversibly bound to the first reagent or the second reagent; or the incubation is carried out in the presence of a further reagent that is reversibly bound to the third receptor-binding agent.

[0042] In some examplesof any of the methods provided herein, the method also includes, after said disruption, further incubating the composition that contains the T cells. In some examples, the incubation and further incubation are carried out in the same vessel; and/or the further incubation is carried out in the presence of the substance; and/or the method does not comprise removing the substance, receptor-binding agent, second receptor-binding agent and/or reagent from the cell composition prior to the further incubation.

[0043] In some examples, the incubation and/or further incubation is carried out at or about 37 °C ± 2 °C; and/or the incubation and/or further incubation is carried out in the presence of a further agent that is capable of delivering a signal to T cells. In some examples, the further agent is capable of enhancing or inducing proliferation of T cells, CD4+ T cells and/or CD8+ T cells. In some examples, the further agent is a cytokine selected from among IL-2, IL-15 and IL-7. In some examples, the further incubation is carried out for a time that is no more than 14 days, no more than 12 days, no more than 10 days, no more than 8 days or no more than 6 days.

[0044] Provided herein are methods for culturing T cells that include: incubating a composition that contains T cells in the presence of a receptor-binding agent that specifically binds to a CD28 molecule on the surface of T cells under conditions to effect signaling through CD28 in the cells; and within 5 days after initiation of said incubation, eliminating or reducing the binding of the receptor-binding agent and the CD28 molecule, whereby the CD28 signaling is terminated or lessened in the cells, thereby generating cultured T cells. In some examples, the elimination or reduction is carried out within 4 days, within 3 days, within 2 days or within 1 day after initiation of the incubation.

[0045] In some instances, the elimination or reduction is effected within 4 days, within 3 days, within 2 days or within 1 day after initiation of the incubation. In some cases, the eliminating or reducing includes washing the cells, whereby any receptor-binding agent that is not specifically bound to CD28 is removed or reduced from the composition. Disclosed is that the eliminating includes reversing the binding interaction between the receptor-binding agent and the CD28 molecule, further including washing the cells to remove or reduce the receptor-binding agent from the composition.

[0046] Disclosed is that during at least a portion of the incubation and/or subsequent to the incubation, T cells in the composition are incubated in the presence of an agent that specifically binds a molecule of the TCR/CD3 complex, whereby a TCR/CD3 complex-associated signal is induced or modulated in the cells.

[0047] Disclosed is a method for culturing T cells including incubating a composition containing T cells in the presence of a receptor-binding agent. In some examples, the receptor-binding agent is reversibly bound to a reagent containing a plurality of binding sites capable of reversibly binding to the receptor-binding agent. In some cases, the receptor-binding agent is capable of specifically binding to a molecule on the surface

of the target cells other than CD28, CD3 or CD40 in a manner that induces or modulates a signal in the target cells and/or alters a function of the target cells, thereby generating cultured target cells. In some examples, the molecule is not CD137 and/or the receptor-binding agent does not specifically bind to CD137.

[0048] Disclosed is that the incubation is performed under conditions in which the receptor-binding agent specifically binds to the molecule, thereby inducing or modulating the signal in the T cells. In some examples, the signal is not a TCR/CD3 complex-associated signal.

[0049] In some examples, the signal is not a TCR/CD3 complex-associated signal.

[0050] In some examples, the receptor-binding agent includes a binding partner C1. In some such examples, the plurality of binding sites of the reagent includes two or more binding sites, Z1, which each are capable of binding to the binding partner C1 to form the reversible bond between the receptor-binding agent and the reagent.

[0051] In some examples, the molecule is selected from among CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40 and HVEM; and/or the receptor-binding agent specifically binds to CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40 or HVEM.

[0052] In some examples, the receptor-binding agent is a second receptor-binding agent and the molecule is a second molecule, and the incubation is further carried out in the presence of a first receptor-binding agent, which is capable of specifically binding to a first molecule on the surface of one or more of the T cells, which first molecule is optionally capable of inducing or modulating a first signal in one or more T cells in the composition. In some examples, the first receptor-binding agent is reversibly bound to the reagent, said reagent that includes a plurality of binding sites for the first receptor-binding agent and the second receptor-binding agent; or the first receptor-binding agent is reversibly bound to a second reagent that includes a plurality of binding sites capable of reversibly binding to the first receptor-binding agent.

[0053] In some examples, the first receptor-binding agent is capable of initiating a TCR/CD3 complex-associated signal in the T cells. Disclosed is that the first receptor-binding agent specifically binds to a member of a TCR/CD3 complex. In some cases, the first receptor-binding agent specifically binds to CD3.

[0054] In some examples, the specific binding of the second receptor-binding agent to the second molecule is capable of enhancing, dampening or modifying a signal delivered through the first molecule. In some cases, the specific binding of the second receptor-binding

agent to the second molecule is capable of enhancing or potentiating a TCR/CD3 complex-associated signal.

[0055] Provided herein in some examplesis a method for culturing T cells, which method includes incubating a composition containing T cells in the presence of a first receptor-binding agent which is reversibly bound to a reagent containing a plurality of binding sites capable of reversibly binding to the first receptor-binding agent. In some cases, first receptor-binding agent is capable of specifically binding to a first molecule on the surface of the T cells, such as to induce or modulate a TCR/CD3 complex-associated signal in T cells in the composition. In some examples, the method includes incubating the composition containing the T cells in the presence of a second receptor-binding agent which may be reversibly bound to the reagent further containing a plurality of binding sites for the second receptor-binding agent or to a second reagent containing a plurality of binding sites capable of reversibly binding to the second receptor-binding agent. Disclosed is that the second receptor-binding agent is capable of specifically binding to a second molecule on the surface of T cells such as to induce or modulate a second signal in T cells in the composition. Disclosed is that the second molecule is other than CD28. In some examples, the incubation is performed under conditions in which the signal and/or second signal are induced or modulated in T cells in the composition, thereby generating cultured T cells.

[0056] In some examples, the first receptor-binding agent specifically binds to a member of a TCR/CD3 complex and/or the first receptor-binding agent specifically binds to CD3.

[0057] In some instances, the specific binding of the second receptor-binding agent to the second molecule is capable of inducing or modulating a signal other than a TCR/CD3 complex-associated signal. Disclosed is that the specific binding of the second receptor-binding agent to the second molecule is capable of enhancing, dampening or modifying a signal delivered through the first molecule. In some examples, the specific binding of the second receptor-binding agent to the second molecule is capable of enhancing or potentiating a TCR/CD3 complex-associated signal.

[0058] In some examples, the molecule, which can be the second molecule, is CD90 (Thy-1), CD95 (Apo-/Fas), CD137, (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40 or HVEM. Disclosed is that the receptor-binding agent, which can be the second receptor-binding agent, specifically binds to CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40 or HVEM. In some examples, the molecule, which can be a

second molecule, is not CD137 and/or the receptor-binding agent, which can be the second receptor-binding agent, does not specifically bind to CD137.

[0059] Disclosed is that the first receptor-binding agent and the second receptor-binding agent reversibly bind to the reagent. In some examples, the first receptor-binding agent and second receptor-binding agent each individually include a binding partner C1, and the plurality of binding sites includes two or more binding site, Z1, which each are capable of binding to the binding partner C1 to form the reversible bond between the first and second receptor-binding agent and the reagent. In other examples, the first receptor-binding agent includes a binding partner C1, the second receptor-binding agent includes a binding partner C2, and the plurality of binding sites includes two or more binding sites, Z1, which each are capable of binding to the binding partner C1 and the binding partner C2 to form the reversible bond between the first and second receptor-binding agent and the reagent. In still further examples, the first receptor-binding agent includes a binding partner C1, the second receptor binding agent includes a binding partner C2, and the plurality of binding sites includes two or more binding site, Z1, which each are capable of binding to the binding partner C1 to form the reversible bond between the first receptor-binding agent and the reagent and two or more binding site, Z2, which each are capable of binding to the binding partner C2 to form the reversible bond between the second receptor-binding agent and the reagent.

[0060] In some examples, the receptor-binding agent specifically binds to a cytokine receptor selected from among IL-2R, IL-1R, IL-15R, IFN-gammaR, TNF-alphaR, IL-4R, IL-10R, Type I IFNR, IL-12R, IL-15R, IL-17R, TNFR1 and TNFR2. In some examples,the receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among IL-2R, IL-1R, IL-15R, IFN-gammaR, TNF-alphaR, IL-4R, IL-10R, Type I IFNR, IL-12R, IL-15R, IL-17R, TNFR1 and TNFR2; and/or the receptor-binding agent is a ligand selected from among IL-2, IL-1, IL-15, IFN-gamma, TNF-alpha, IL-4, IL-10, IL-12, IL-15, IL-17 and TNF, or is a biologically active fragment thereof. In some examples,the receptor-binding agent specifically binds to a cytokine receptor selected from among IL-12R, IFN-gammaR, IL-4R and IL-17R; or the receptor-binding agent is a ligand selected from among IL-2, IL-4, IL-7, IL-10, IL-15 and IL-17 or is a biologically active fragment thereof.

[0061] In some examples, the receptor-binding agent specifically binds to a cytokine receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain). In some examples, the receptor-binding agent is a ligand that specifically binds to a cytokine

receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain); and/or the receptor-binding agent is a ligand selected from among IL-7, IL-21, IL-2, IL-4, IL-9 and IL-15, or is a biologically active fragment thereof. In some examples,the receptor-binding agent specifically binds to a cytokine receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain); or the receptor-binding agent is a ligand selected from among IL-7, IL-21, IL-2, IL-4, IL-9 and IL-15, or is a biologically active fragment thereof.

[0062] In some examples, the receptor-binding agent specifically binds to a chemokine receptor selected from among CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR9, CXCR1, CXCR3 and CXCR4. In some examples,the receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR9, CXCR1, CXCR3 and CXCR4; or the receptor-binding agent is a ligand selected from among CXCL9, CXCL10, CCL19, CCL21 and CCL25 or is a biologically active fragment thereof. In some examples, the receptor-binding agent specifically binds to a chemokine receptor selected from among CXCR3, CCR7, CXCR1 and CXCR4; or the receptor-binding agent is a ligand selected from among CXCL9, CXCL10, CCL19, CCL21 and CCL25 or is a biologically active fragment thereof.

[0063] In some examples, the adhesion molecule is selected from among CD44, CD31, CD18/CD11a (LFA-1), CD29, CD54 (ICAM-1), CD62L (L-selectin), and CD29/CD49d (VLA-4), CD106 (VCAM-1) or is a biologically active fragment thereof. In some examples, the adhesion molecule is selected from among LFA-1, L-selectin, VCAM-1 and VLA-4 or is a biologically active fragment thereof.

[0064] In some examples, the factor is a nuclear factor. In some examples, the factor is a retinoic acid receptor-related orphan receptor gamma (RORgamma) or RORalpha.

[0065] In some examplesof any of the methods provided herein, the incubation is performed under conditions in which the receptor-binding agent specifically binds to the molecule, thereby inducing or modulating the signal in the target cells and/or altering a function in the target cells. In some examples, the receptor-binding agent includes a binding partner C1, and the plurality of binding sites includes two or more binding site, Z1, which each are capable of binding to the binding partner C1 to form the reversible bond between the receptor-binding agent and the reagent. In some examples, the receptor-binding agent is an additional receptor-binding agent and the molecule is an additional molecule, and the incubation is further carried out in the presence of a first receptor-binding agent, which is capable of specifically

binding to a first molecule on the surface of one or more of the T cells, which first molecule is optionally capable of inducing or modulating a first signal in one or more T cells in the composition.

[0066] In some examples, the first receptor-binding agent is reversibly bound to the reagent, said reagent that includes a plurality of binding sites for the first receptor-binding agent and the additional receptor-binding agent; or the first receptor-binding agent is reversibly bound to a second reagent that includes a plurality of binding sites capable of reversibly binding to the first receptor-binding agent. In some examples, the first receptor-binding agent is capable of initiating a TCR/CD3 complex-associated signal in the T cells; and/or the first receptor-binding agent specifically binds to a member of a TCR/CD3 complex; and/or the first receptor-binding agent specifically binds to CD3.

[0067] In some examples, the first receptor-binding agent includes a binding partner C2, and the plurality of binding sites includes two or more binding site, Z2, which each are capable of binding to the binding partner C2 to form the reversible bond between the first receptor-binding agent and the reagent.

[0068] In some examples, the incubation is further carried out in the presence of a second receptor-binding agent, which is capable of specifically binding to a second molecule on the surface of one or more of the T cells, which second molecule is optionally capable of inducing or modulating a signal in target cells in the composition to enhance, dampen or modify a signal delivered through the first molecule.

[0069] In some examples, the second signal is a signal other than a TCR/CD3 complex-associated signal; the second signal is capable of enhancing or potentiating a TCR/CD3 complex-associated signal; or the second molecule is a costimulatory molecule, accessory molecule, cytokine receptor, chemokine receptor, immune checkpoint molecule or is a member of the TNF family or the TNF receptor family. In some examples, the second molecule is selected from among CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40 and HVEM; and/or the second receptor-binding agent specifically binds to CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40 or HVEM. In some examples, the second molecule is selected from among CD28 and CD137. In some examples, the second molecule is CD28.

[0070] Provided herein are methods for culturing target cells, which method includes incubating a composition containing target cells in the presence of a receptor-binding agent.

The receptor-binding agent may be reversibly bound to a reagent that is a streptavidin analog or mutein containing a plurality of binding sites capable of reversibly binding to the receptor-binding agent. In some cases, the receptor-binding agent is capable of specifically binding to a molecule on the surface of the target cells, such as to induce or modulate a signal in target cells in the composition. Disclosed is that the mutein streptavidin includes a net negative charge or exhibits an isoelectric point less than the streptavidin mutein comprising the sequence set forth in SEQ ID NO:4 or 6, thereby generating cultured target cells.

[0071] Disclosed is a method for culturing target cells, the method including incubating a composition containing target cells in the presence of a receptor-binding agent that is reversibly bound to a reagent that is a streptavidin analog or mutein containing a plurality of binding sites capable of reversibly binding to the receptor-binding agent. In some examples, the receptor-binding agent is capable of specifically binding to a molecule on the surface of the target cells in a manner that induces or modulates a signal in target cells in the composition. In some cases, the streptavidin analog or mutein exhibits a higher affinity for a streptavidin-binding peptide containing the sequence of amino acids Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8) than a streptavidin or mutein containing the sequence of amino acids set forth in any of SEQ ID NOS: 1-6, thereby generating cultured target cells.

[0072] In some examples, the incubation is performed under conditions in which the receptor-binding agent specifically binds to the molecule, thereby inducing or modulating the signal in one or more target cells in the composition.

[0073] In some instances, the plurality of binding sites of the reagent includes two or more binding sites, Z1. In some cases, the receptor-binding agent includes a binding partner C1, which is capable of reversibly binding to the binding site Z1, wherein the reversible binding between C1 and Z1 effects the reversible binding between the receptor-binding agent and the reagent. In some examples, the streptavidin analog or mutein includes a plurality of binding sites Z1, and a plurality of receptor-binding agents are reversibly bound to the reagent.

[0074] In some examples, the target cells comprise immune cells. For example, in some examples, the target cells comprise blood cells; the target cells comprise leukocytes; the target cells comprise lymphocytes; the target cells comprise B cells; the target cells comprise a B cell population; the target cells comprise T cells; the target cells comprise a T cell population; and/or the target cells comprise natural killer (NK) cells. In some examples, the target cells comprise antigen-specific T cells or a population thereof, a T helper cell or population thereof, a

cytotoxic T cell or population thereof, a memory T cell or population thereof, a regulatory T cell or population thereof, an NK cell or population thereof, antigen-specific B cells or a population thereof, a memory B cell or population thereof, or a regulatory B cell or population thereof. In some examples, the target cells are T cells.

[0075] In some examples, the molecule is present on the surface of T cells, and the receptor-binding agent is capable of inducing or modulating a signal in T cells in the composition. Disclosed is that the receptor-binding agent is capable of initiating a TCR/CD3 complex-associated signal in the T cells and/or the receptor-binding agent specifically binds to a member of a TCR/CD3 complex. In some cases, the receptor-binding agent, specifically binds to CD3. In some instances, the molecule is a first molecule and the receptor-binding agent is capable of specifically binding to the first molecule and, in some cases, a second molecule on the surface of one or more of the target cells. In some examples, binding to the second molecule is capable of enhancing, dampening, or modifying a signal delivered through the first molecule.

[0076] In some examples, the molecule is a first molecule and the receptor-binding agent is capable of specifically binding to the first molecule and a second molecule on the surface of one or more of the target cells, which binding to the second molecule induces or modulates a signal in the target cells. In some examples, binding to the second molecule is optionally capable of enhancing, dampening, or modifying a signal delivered through the first molecule.

[0077] , the receptor-binding agent is a first receptor-binding agent and the incubation is further carried out in the presence of a second receptor-binding agent. The second receptor-binding agent may be capable of specifically binding to a second molecule on the surface of one or more of the target cells. In some cases, binding of the second receptor-binding agent to the second molecule is capable of inducing or modulating a signal to enhance, dampen, or modify a signal delivered through the first molecule.

[0078] In some examples, the incubation is performed under conditions in which the second receptor-binding agent specifically binds to the second molecule, thereby inducing or modulating a signal in target cells in the composition to enhance, dampen or modify a signal delivered through the first molecule. In some examples, the streptavidin mutein or analog includes a plurality of binding sites capable of reversibly binding to the second receptor-binding

agent, whereby the second receptor-binding agent is reversibly bound to the streptavidin mutein or analog.

[0079] In some examples, the second receptor-binding agent includes a binding partner C1 or C2, which is capable of reversibly binding to the two or more binding sites Z2 present in the streptavidin analog or mutein.

[0080] In some cases, the additional molecule is CD28, CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40 or HVEM. In some examples, the second receptor-binding agent specifically binds to CD28, CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40 or HVEM. Disclosed is that the additional molecule is CD40 and CD137. In some instances, the second receptor-binding agent specifically binds to CD40 or CD137.

[0081] In some examples, the second molecule is or includes an adhesion molecule or is a factor that induces cytokine production, chemokine production and/or expression of an adhesion molecule.

[0082] In some examples, the second receptor-binding agent specifically binds to a cytokine receptor selected from among IL-2R, IL-1R, IL-15R, IFN-gammaR, TNF-alphaR, IL-4R, IL-10R, Type I IFNR, IL-12R, IL-15R, IL-17R, TNFR1 and TNFR2. In some examples, the second receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among IL-2R, IL-1R, IL-15R, IFN-gammaR, TNF-alphaR, IL-4R, IL-10R, Type I IFNR, IL-12R, IL-15R, IL-17R, TNFR1 and TNFR2; and/or the second receptor-binding agent is a ligand selected from among IL-2, IL-1, IL-15, IFN-gamma, TNF-alpha, IL-4, IL-10, IL-12, IL-15, IL-17 and TNF, or is a biologically active fragment thereof. In some examples, the second receptor-binding agent specifically binds to a cytokine receptor selected from among IL-12R, IFN-gammaR, IL-4R and IL-17R; or the second receptor-binding agent is a ligand selected from among IL-2, IL-4, IL-7, IL-10, IL-15 and IL-17 or is a biologically active fragment thereof.

[0083] In some examples, the second receptor-binding agent specifically binds to a cytokine receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain). In some examples, the second receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain); and/or the second receptor-binding agent is a ligand selected from among IL-7, IL-21, IL-2, IL-4, IL-9 and IL-15, or is a biologically active fragment thereof. In some examples, the second receptor-binding agent specifically binds to a cytokine receptor

selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain); or the second receptor-binding agent is a ligand selected from among IL-7, IL-21, IL-2, IL-4, IL-9 and IL-15, or is a biologically active fragment thereof.

[0084] In some examples, the second receptor-binding agent specifically binds to a chemokine receptor selected from among CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR9, CXCR1, CXCR3 and CXCR4. In some examples, the second receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR9, CXCR1, CXCR3 and CXCR4; or the second receptor-binding agent is a ligand selected from among CXCL9, CXCL10, CCL19, CCL21 and CCL25 or is a biologically active fragment thereof. In some examples, the second receptor-binding agent specifically binds to a chemokine receptor selected from among CXCR3, CCR7, CXCR1 and CXCR4; or the second receptor-binding agent is a ligand selected from among CXCL9, CXCL10, CCL19, CCL21 and CCL25 or is a biologically active fragment thereof.

[0085] In some examples, the adhesion molecule is selected from among CD44, CD31, CD18/CD11a (LFA-1), CD29, CD54 (ICAM-1), CD62L (L-selectin), and CD29/CD49d (VLA-4), CD106 (VCAM-1) or is a biologically active fragment thereof. In some examples, the adhesion molecule is selected from among LFA-1, L-selectin, VCAM-1 and VLA-4 or is a biologically active fragment thereof.

[0086] In some examples, the factor is a nuclear factor. In some examples, the factor is a retinoic acid receptor-related orphan receptor gamma (RORgamma) or RORalpha.

[0087] In some examples, the second receptor-binding agent includes a plurality of different receptor-binding agents, each of which is capable of individually binding to the same or different second molecule on the surface of T cells in the composition to collectively induce or modulate one or more signals in the cells.

[0088] In some examples, the method further includes disrupting the reversible binding between the first and/or second receptor-binding agent and the reagent. Disclosed is that the disruption is effected within 14 days after initiation of the incubation, within 12 days after initiation of the incubation, within 10 days after initiation of the incubation within 8 days after initiation of the incubation or within 6 days after initiation of the incubation.

[0089] In some examples, at least a portion of the incubation is carried out in the presence of a further agent that is capable of delivering a signal to T cells. In some cases, the further agent is capable of enhancing or inducing proliferation of T cells, CD4+ T cells and/or

CD8+ T cells. In some examples, the further agent is a cytokine such as IL-2, IL-15 or IL-7. In some instances, the further agent does not specifically bind to CD28 and/or induce CD28 signaling.

[0090] In some examples, the T cells or target cells are primary cells from a subject. In some cases, the T cells or target cells are directly isolated from a subject. In some examples, the T cells are unfractionated T cells, are enriched or isolated CD3+ T cells, are enriched or isolated CD4+ T cells, or are enriched or isolated CD8+ T cells. In some examples, prior to the incubating the T cells are not enriched for CD62L+ cells and/or are not enriched for naïve T cells. In some cases, the T cells or target cells are human cells.

[0091] Disclosed is that the reagent has a size that is less than 20 nm, less than 10 nm, less than 5 nm or less than 1 nm. In some instances, the reagent has a density of less than 1.2 g/cm3 or less than 1.0 g/cm3.

[0092] In some examples, the reagent is not, and is not bound to or associated with, a solid support, stationary phase, a bead, a microparticle, a magnetic particle, and/or a matrix during said incubation; and/or the reagent is flexible, does not contain a metal or magnetic core, is comprised entirely or primarily of organic multimer, is not spherical, is not substantially spherical or uniform in shape and/or is not rigid.

[0093] In some examples, the reagent is not bound to a support or a solid support during said incubation. In some examples, the reagent is bound to a support during at least a portion of the incubation, whereby a plurality of the T cells or target cells are reversibly immobilized on the support during at least a portion of the incubation. In some examples, the support is or includes a stationary phase; and/or the support is or includes a solid support.

[0094] In some examples, the receptor-binding agent, which can be the first receptor-binding agent, contains only one binding site, such as B2. Disclosed is that the receptor-binding agent, which can be the first receptor-binding agent, specifically binds to the molecule in a monovalent manner. In some examples, the second receptor-binding agent contains only one binding site, such as B4. In some cases, the second receptor-binding agent specifically binds to the molecule in a monovalent manner. In some examples, the binding site, such as B2 or B4, contains an antibody combining site.

[0095] Disclosed is that the receptor-binding agent, which can be a first receptor-binding agent, and/or the second receptor-binding agent each individually is an antibody fragment, a monovalent antibody fragment, a proteinaceous binding molecule with antibody-like binding

properties, a molecule containing Ig domains, a cytokine, a chemokine, an aptamer, or MHC molecule or binding fragments thereof. Disclosed is that the receptor-binding agent, which can be the first receptor-binding agent, and/or the second receptor-binding agent include an antibody fragment, a Fab fragment, a divalent antibody fragment such as a $F(ab')_2$-fragment or a divalent single-chain Fv (scFv) fragment. In some cases, the receptor-binding agent, which can be the first receptor-binding agent, and/or the second receptor-binding agent is a monovalent antibody fragment such as a Fab fragment, an Fv fragment or an scFv fragment. In some instances, the receptor-binding agent, which can be the first receptor-binding agent, and/or the second receptor-binding agent is a proteinaceous binding molecule with antibody-like binding properties, such as an aptamer, mutein based on a polypeptide of the lipocalin family, glubody, protein based on the ankyrin scaffold, protein based on the crystalline scaffold, adnectin or an avimer.

[0096] In some examples, the receptor-binding agent, which can be the first receptor-binding agent, includes an agent that specifically binds to CD3. The agent that specifically binds CD3 can be an anti-CD3-antibody, a divalent antibody fragment of an anti-CD3 antibody, a monovalent antibody fragment of an anti-CD3-antibody, or a proteinaceous CD3 binding molecule with antibody-like binding properties. In some examples, the second receptor-binding agent includes an agent that specifically binds to CD28, CD90, CD95, CD137, CD154, ICOS, LAT, CD27, OX40 and/or HVEM. The agent that specifically binds to CD28, CD90, CD95, CD137, CD154, ICOS, LAT, CD27, OX40 and/or HVEM can be an anti-CD28-antibody, a divalent antibody fragment of an anti-CD28 antibody, an antibody fragment of an anti-CD28-antibody, a proteinaceous CD28 binding molecule with antibody-like binding properties, an anti-CD90-antibody, a divalent antibody fragment of an anti-CD90 antibody, an antibody fragment of an anti-CD90-antibody, a proteinaceous CD90 binding molecule with antibody-like binding properties, an anti-CD95-antibody, a divalent antibody fragment of an anti-CD95 antibody, an antibody fragment of an anti-CD95-antibody, a proteinaceous CD95 binding molecule with antibody-like binding properties, an anti-CD154-antibody, a divalent antibody fragment of an anti-CD154 antibody, a monovalent antibody fragment of an anti-CD154-antibody, a proteinaceous CD154 binding molecule with antibody-like binding properties, an anti-CD137-antibody, a divalent antibody fragment of an anti-CD137 antibody, a monovalent antibody fragment of an anti-CD137-antibody, a proteinaceous CD137 binding molecule with antibody-like binding properties, an anti-ICOS-antibody, a divalent antibody fragment of an anti-ICOS

antibody, an antibody fragment of an anti-ICOS-antibody, a proteinaceous ICOS binding molecule with antibody-like binding properties, an anti-LAT-antibody, a divalent antibody fragment of an anti-LAT antibody, an antibody fragment of an anti-LAT-antibody, a proteinaceous LAT binding molecule with antibody-like binding properties, an anti-CD27-antibody, a divalent antibody fragment of an anti-CD27 antibody, an antibody fragment of an anti-CD27-antibody, a proteinaceous CD27 binding molecule with antibody-like binding properties, an anti-OX40-antibody, a divalent antibody fragment of an anti-OX40 antibody, an antibody fragment of an anti-OX40-antibody, a proteinaceous OX40 binding molecule with antibody-like binding properties, an anti-HVEM-antibody, a divalent antibody fragment of an anti-HVEM antibody, an antibody fragment of an anti-HVEM-antibody, a proteinaceous HVEM binding molecule with antibody-like binding properties, 4-1BB ligand, or any mixture thereof.

[0097] In some examplesof any of the methods provided herein, the receptor-binding agent comprise an antibody fragment; the receptor-binding agent comprise a Fab fragment; the receptor-binding agent is a divalent antibody fragment selected from among a $F(ab')_2$-fragment and a divalent single-chain Fv (scFv) fragment; the receptor-binding agent is a monovalent antibody fragment selected from among a Fab fragment, an Fv fragment and an scFv fragment; and/or the receptor-binding agent is a proteinaceous binding molecule with antibody-like binding properties selected from among aptamers, muteins based on a polypeptide of the lipocalin family, glubodies, proteins based on the ankyrin scaffold, proteins based on the crystalline scaffold, adnectins and avimers.

[0098] In some examplesof any of the methods provided herein, the reagent is or contains streptavidin, avidin, an analog or mutein of streptavidin that reversibly binds biotin, a biotin analog or a biologically active fragment thereof; an analog or mutein of avidin or streptavidin that reversibly binds a streptavidin-binding peptide; a reagent that includes at least two chelating groups K, wherein the at least two chelating groups are capable of binding to a transition metal ion; an agent capable of binding to an oligohistidine affinity tag; an agent capable of binding to a glutathione-S-transferase; calmodulin or an analog thereof; an agent capable of binding to calmodulin binding peptide (CBP); an agent capable of binding to a FLAG-peptide; an agent capable of binding to an HA-tag; an agent capable of binding to maltose binding protein (MBP); an agent capable of binding to an HSV epitope; an agent capable of binding to a myc epitope; or an agent capable of binding to a biotinylated carrier protein.

[0099] In some examples, the reagent is or contains a streptavidin analog or mutein or an avidin analog or mutein that reversibly binds to biotin or a biologically active fragment; a streptavidin analog or mutein or an avidin analog or mutein that reversibly binds to a biotin analog or a biologically active fragment; and/or a streptavidin analog or mutein or an avidin analog or mutein that reversibly binds to a streptavidin-binding peptide.

[0100] In some examples, the reagent is an oligomer or polymer of streptavidin, avidin, an analog or mutein of streptavidin that reversibly binds biotin or a biologically active fragment; a streptavidin or avidin analog or mutein that reversibly binds a streptavidin-binding peptide; a reagent that includes at least two chelating groups K, wherein the at least two chelating groups are capable of binding to a transition metal ion; an agent capable of binding to an oligohistidine affinity tag; an agent capable of binding to a glutathione-S-transferase; calmodulin or an analog thereof; an agent capable of binding to calmodulin binding peptide (CBP); an agent capable of binding to a FLAG-peptide; an agent capable of binding to an HA-tag; an agent capable of binding to maltose binding protein (MBP); an agent capable of binding to an HSV epitope; an agent capable of binding to a myc epitope; or an agent capable of binding to a biotinylated carrier protein. In some examples, the reagent includes an oligomer or polymer of streptavidin, avidin, a streptavidin analog or mutein or and an avidin analog or mutein. Disclosed is that individual molecules of the oligomer or polymer are crosslinked by a polysaccharide or a bifunctional linker.

[0101] In some examples, the plurality of binding sites Z include at least 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 72 or more binding sites.

[0102] Disclosed is that the streptavidin-binding peptide is Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)3-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys ((SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)2-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) or Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)2Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

[0103] In some examples, the reagent includes a streptavidin analog or mutein containing the amino acid sequence Va144-Thr45-Ala46-Arg47 or lle44-Gly45-Ala46-Arg47 at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO: 1. In some examples, the streptavidin analog or mutein includes the amino acid sequence Va144-Thr45-Ala46-Arg47 at

sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

[0104] In some examples, the streptavidin analog or mutein includes the sequence of amino acids set forth in any of SEQ ID NOS: 3-6. Disclosed is that the streptavidin analog or mutein includes a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS:3-6 and contains the amino acid sequence corresponding to Val44-Thr45-Ala46-Arg47 or Ile44-Gly45-Ala46-Arg47. In some examples, the streptavidin analog or mutein reversibly binds to biotin or a biologically active form thereof, a biotin analog or mutein or a biologically active fragment thereof or a streptavidin-binding peptide. In some examples, the streptavidin analog or mutein binds a functional fragment of any of the above sequences that reversibly binds to biotin or a biologically active form thereof, a biotin analog or mutein or a biologically active fragment thereof or a streptavidin-binding peptide.

[0105] In some examples, the streptavidin analog or mutein further contains an amino acid replacement or replacements at a position corresponding to 117, 120 and/or 121 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO: 1. In some examples, the amino acid replacement or replacements are selected from among Glu117, Asp117, Arg117, Ser120, Ala120, Gly120, Trp121, Tyr121 or Phe121. In some examples, the amino acid replacement or replacements are Glu117, Gly120 or Tyr121.

[0106] In some examples, the streptavidin analog or mutein contains the sequence of amino acids set forth in SEQ ID NO: 27 or 28. Disclosed is that the streptavidin analog or mutein contains a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NOS:28 and contains the amino acid sequence corresponding to Val44, Thr45, Ala46, Arg47, Glu117, Gly120 and Tyr121. In some examples, the streptavidin analog or mutein reversibly binds to biotin or a biologically active fragment, a biotin analog or mutein or a biologically active fragment thereof or a streptavidin-binding peptide. In some examples, the streptavidin analog or mutein reversibly binds a functional fragment any of the above sequences that reversibly binds to biotin or a biologically active fragment, a biotin analog or mutein or a biologically active fragment thereof or a streptavidin-binding peptide.

[0107] In some examples, the binding partner C1 and/or the binding partner C2, independently, includes a streptavidin-binding peptide such as Trp-Ser-His-Pro-Gln-Phe-Glu-

Lys (SEQ ID NO: 8), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)3-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys ((SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)2-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) or Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)2Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19);

[0108] In some examples, the disruption includes introducing to the cells a composition containing a substance capable of reversing the bond between the receptor-binding agent, which can be the first receptor-binding agent, and/or the second-receptor-binding agent and the reagent. In some examples, the substance is a free binding partner and/or is a competition agent. In some examples, the substance in the composition is not detrimental to the T cells or to the target cells. Disclosed is that the addition of the substance does not reduce the percentage of surviving T cells or target cells to less than 90 %, 80 %, 70 %, 60 %, or 50 %, as compared to incubation of the T cells or target cells, respectively, under comparable or the same conditions, without the substance. In some examples, the disruption terminates or lessens the signal induced or modulated by one or both of the receptor-binding agent or second receptor-binding agent in the T cells or the target cells.

[0109] In some examples, the reagent is or contains a streptavidin, avidin, a streptavidin analog or mutein or and an avidin analog or mutein or biologically active fragments thereof. In some examples, the substance contains a streptavidin-binding peptide, biotin or a biologically active fragment, optionally a D-biotin, or a biotin analog or biologically active fragment.

[0110] In some examples, the substance is a streptavidin-binding peptide such as Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)3-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys ((SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)2-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) or Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(GlyGlyGlySer)2Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19). In some examples, the substance is C1 or an analog thereof or is C2 or an analog thereof.

[0111] In some examples, the dissociation constant (K_D) for the reversible binding between the binding site Z1 and the binding partner C1 and/or for the reversible binding between the binding site Z2 and the binding partner C2 is in the range of 10-2 M to 10-13 M.

[0112] In some examples, prior to the incubation, cells are contacted with a selection agent that specifically binds to a marker contained by T cells or target cells of the composition,

thereby generating or obtaining the composition containing the T cells or target cells. In some examples, at least a portion of the incubation is carried out in the presence of a selection agent that specifically binds to a marker comprised by T cells or target cells of the composition, and the cultured T cells are enriched for T cells or target cells containing the marker.

[0113] In some examples, the selection agent is reversibly bound to the reagent, and the reagent further contains a plurality of binding sites capable of specifically binding the selection agent. Disclosed is that the selection agent is reversibly bound to a second reagent containing a plurality of binding sites capable of specifically binding to the selection agent.

[0114] In some examples, the induction or modulation of the signal effects an increase in expansion (proliferation) and/or activation of the cultured T cells compared to incubation of T cells in the absence of the induction or modulation of the signal. In some examples, the increase is at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold or greater. In some examples, the induction or modulation of the additional or second signal increases expansion (proliferation) and/or activation of the T cells compared to incubation of T cells in the absence of the induction or modulation of the additional or second signal. In some examples, the increase is at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold or greater.

[0115] In some examples, the method increases expansion and/or proliferation of T cells in the composition, alters the metabolic profile of T cells in the composition, alters the subset of CD8+ T cells in the composition; and/or increases the percentage of long-lived memory T cells in the composition, each compared to the T cells in the composition prior to the incubation, subsequent to the incubation but in the absence of the disruption or subsequent to the incubation but in the presence of an agent that specifically binds CD28 and/or induces or modulates CD28 signaling.

[0116] In some examples, the methods result in cultured T cells in which the number or percentage of CD3+ T cells, CD4+ T cells or CD8+ cells is increased at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold or 10-fold compared to the number or percentage of CD3+ T cells, CD4+ T cells or CD8+ T cells, respectively, in the composition prior to the incubation, subsequent to the incubation but in the absence of the disruption, or subsequent to an analogous incubation but performed in the presence of an agent that specifically binds CD28 and/or induces or modulates CD28 signaling. In some examples, the methods result in cultured T cells in which the ratio of CD8+ T cells or the relative or normalized ratio of CD8+ T

cells in the composition is increased at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold or 10-fold compared to the ratio or the relative or normalized ratio of CD8+ T cells in the composition prior to the incubation, subsequent to the incubation but in the absence of the disruption, or subsequent to an analogous incubation but in the presence of an agent that specifically binds CD28 and/or induces or modulates CD28 signaling. In some examples, the methods result in cultured T cells in which the number or percentage of CD62L+, optionally long-lived memory T cells or memory stem cells (T_{SCM}), in the composition is increased at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold or 10-fold compared to the number or percentage of the corresponding population of cells, either CD62L+, long-lived memory T cells or T_{SCM}, in the composition prior to the incubation, subsequent to the incubation but in the absence of the disruption, or subsequent to an analogous incubation but in the presence of an agent that specifically binds CD28 and/or induces or modulates CD28 signaling.

[0117] In some examples, the cultured T cells contain greater than 35%, 40%, 45%, 50%, 60%, 70%, 80% or 90% of a T cell subset containing a phenotype that is surface positive for CD62L (CD62L+) as a percentage of the total T cells in the composition or the total cells in the composition.

[0118] In some examples, the T cell subset further includes a phenotype including CD127+; and/or any one or more of CD45RA+, CD45RO-, CCR7+ and CD27+ and any one or more of t-bet^{low}, IL-7Ra+, CD95+, IL-2Rβ+, CXCR3+ and LFA-1+.

[0119] In some examples, the T cell subset includes a low level of TCR rearrangement excisions circles (TREC); and/or expresses a proliferation marker, which can be Ki-67; and/or exhibits the capacity to proliferate in the presence of a stimulatory agent; and/or exhibits the capacity to produce a cytokine such as IFN-gamma, TNF or IL-2 in the presence of a stimulatory agent.

[0120] In some examples, the stimulatory agent is an antigen, a homeostatic cytokine, such as IL-15 and/or IL-17, or is an agent that is capable of initiating a TCR/CD3 complex-associated signal in the T cells.

[0121] In some examples, the T cell subset is or contains long-lived memory T cells. In some examples, the T cell subset is or includes T memory stem cells (T_{SCM}).

[0122] In some examples, the method further includes introducing a recombinant nucleic acid molecule into T cells or target cells of the population. Disclosed is that the nucleic acid molecule may encode a recombinant protein, whereby cells express the recombinant

protein. In some examples, the recombinant receptor is a chimeric antigen receptor or transgenic T cell receptor (TCR). Disclosed is that the method is performed *in vitro* or *ex vivo*.

[0123] In some examples, the method further includes administering the cultured cells to a subject having a disease or condition.

[0124] Disclosed is a composition containing a plurality of cultured T cells or target cells produced by the methods provided herein, and optionally a pharmaceutically acceptable excipient.

[0125] In some examples, after addition of the substance, the cells have not been incubated *in vitro* or *ex vivo* at a temperature greater than 30 °C for more than 24 hours, more than 48 hours , more than 72 hours or more than 96 hours.

[0126] Also provided herein are articles of manufacture that include: a) a reagent that includes a plurality of binding sites capable of binding to a receptor-binding agent; and b) the receptor-binding agent which i) is reversibly bound to the reagent and ii) is capable of specifically binding to a molecule on the surface of T cells in a manner that induces or modulates a signal in T cells. In some examplesmolecule is not CD28, CD3, or CD40.

[0127] In some examples, binding the molecule induces or modulates a signal in a T cell other than a TCR/CD3 complex-associated signal; and/or binding the molecule enhances or potentiates a TCR/CD3 complex-associated signal. In some examples, the molecule is selected from among CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40 and HVEM. In some examples, the molecule is not CD137.

[0128] In some examples, the receptor-binding agent specifically binds to a cytokine receptor, specifically binds to a chemokine receptor, is or includes an adhesion molecule or is a factor that induces cytokine production, chemokine production and/or expression of an adhesion molecule.

[0129] In some examples, the receptor-binding agent specifically binds to a cytokine receptor selected from among IL-2R, IL-1R, IL-15R, IFN-gammaR, TNF-alphaR, IL-4R, IL-10R, Type I IFNR, IL-12R, IL-15R, IL-17R, TNFR1 and TNFR2. In some examples, the receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among IL-2R, IL-1R, IL-15R, IFN-gammaR, TNF-alphaR, IL-4R, IL-10R, Type I IFNR, IL-12R, IL-15R, IL-17R, TNFR1 and TNFR2; and/or the receptor-binding agent is a ligand selected from among IL-2, IL-1, IL-15, IFN-gamma, TNF-alpha, IL-4, IL-10, IL-12, IL-15, IL-17 and TNF, or is a biologically active fragment thereof. In some examples, the receptor-

binding agent specifically binds to a cytokine selected from among IL-12R, IFN-gammaR, IL-4R and IL-17R; or the receptor-binding agent is a ligand selected from among IL-2, IL-4, IL-7, IL-10, IL-15 and IL-17 or is a biologically active fragment thereof.

[0130] In some examples, the second receptor-binding agent specifically binds to a cytokine receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain). In some examples, the second receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain); and/or the second receptor-binding agent is a ligand selected from among IL-7, IL-21, IL-2, IL-4, IL-9 and IL-15, or is a biologically active fragment thereof. In some examples, the second receptor-binding agent specifically binds to a cytokine receptor selected from among IL-7R, IL-21R and CD132 (IL receptor common gamma chain); or the second receptor-binding agent is a ligand selected from among IL-7, IL-21, IL-2, IL-4, IL-9 and IL-15, or is a biologically active fragment thereof.

[0131] In some examples, the receptor-binding agent specifically binds to a chemokine receptor selected from among CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR9, CXCR1, CXCR3 and CXCR4. In some examples, the receptor-binding agent is a ligand that specifically binds to a cytokine receptor selected from among CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR9, CXCR1, CXCR3 and CXCR4; or the receptor-binding agent is a ligand selected from among CXCL9, CXCL10, CCL19, CCL21 and CCL25 or is a biologically active fragment thereof. In some examples, the receptor-binding agent specifically binds to a cytokine selected from among CXCR3, CCR7, CXCR1 and CXCR4; or the receptor-binding agent is a ligand selected from among CXCL9, CXCL10, CCL19, CCL21 and CCL25 or is a biologically active fragment thereof.

[0132] In some examples, the adhesion molecule is selected from among CD44, CD31, CD18/CD11a (LFA-1), CD29, CD54 (ICAM-1), CD62L (L-selectin), and CD29/CD49d (VLA-4), CD106 (VCAM-1) or is a biologically active fragment thereof. In some examples, the adhesion molecule is selected from among LFA-1, L-selectin, VCAM-1 and VLA-4 or is a biologically active fragment thereof.

[0133] In some examples, the factor is a nuclear factor. In some examples, the factor is a retinoic acid receptor-related orphan receptor gamma (RORgamma) or RORalpha.

[0134] Provided herein are methods for culturing target cells that include incubating a composition that contains target cells in the presence of one or more receptor-binding agent,

wherein the receptor-binding agent reversibly bound to a reagent that is a streptavidin analog or mutein containing a plurality of binding sites capable of binding to an agent. Disclosed is that the streptavidin analog or mutein includes a net negative charge or exhibits an isoelectric point less than the streptavidin mutein comprising the sequence of amino acids set forth in SEQ ID NO:4 or 6 and/or exhibits a higher affinity for a streptavidin-binding peptide containing the sequence of amino acids Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8) than a streptavidin or mutein containing the sequence of amino acids set forth in any of SEQ ID NOS: 1-6. In some examples, the agent is reversibly bound to the reagent and is capable of specifically binding to a molecule on the surface of a cell.

[0135] In some examples, the receptor-binding agent includes a binding partner C1. Disclosed is that the plurality of binding sites includes two or more binding sites, Z1, which each are capable of binding to the binding partner C1 to form the reversible bond between the receptor-binding agent or agent and the reagent.

[0136] In some examples, the agent is a selection agent or a receptor-binding agent.

[0137] In some examples, the agent is a receptor-binding agent that specifically binds to a molecule that is or includes a member of a TCR/CD3 complex, CD3, a costimulatory molecule, accessory molecule, cytokine receptor, chemokine receptor, immune checkpoint molecule or is a member of the TNF family or the TNF receptor family or is or includes an adhesion molecule or is a factor that induces cytokine production, chemokine production and/or expression of an adhesion molecule.

[0138] In some examples, the receptor-binding agent is a second receptor-binding agent and the molecule is a second molecule, and the reagent further includes: c) a plurality of binding sites capable of reversibly binding to a first receptor-binding agent and d) the first receptor-binding agent which i) is reversibly bound to the reagent and ii) is capable of specifically binding to a first molecule on the surface of a T cell.

[0139] In some examples, the agent or the first receptor-binding agent specifically binds to a member of a TCR/CD3 complex and/or the first receptor-binding agent specifically binds to CD3.

[0140] In some examples, the first receptor-binding agent and second receptor-binding agent each individually contains a binding partner C1, and the plurality of binding sites includes two or more binding site, Z1, which each are capable of binding to the binding partner C1 to form the reversible bond between the first and second receptor-binding agent and the reagent.

Disclosed is that the first receptor-binding agent contains a binding partner C1, the second receptor-binding agent contains a binding partner C2, and the plurality of binding sites include two or more binding sites, Z1, which each are capable of binding to the binding partner C1 and the binding partner C2 to form the reversible bond between the first and second receptor-binding agent and the reagent. In some examples, the first receptor-binding agent contains a binding partner C1, the second receptor binding agent contains a binding partner C2, and the plurality of binding sites includes two or more binding sites, Z1, which each are capable of binding to the binding partner C1 to form the reversible bond between the first receptor-binding agent and the reagent and two or more binding sites, Z2, which each are capable of binding to the binding partner C2 to form the reversible bond between the second receptor-binding agent and the reagent.

[0141] In some examples, the reagent has a size that is less than 20 nm, less than 10 nm, less than 5 nm or less than 1 nm. In some examples, the reagent has a density of less than 1.2 g/cm3 or less than 1.0 g/cm3. In some examples, the reagent is not bound to a support or solid support. In some instances, the reagent is bound or immobilized to a support. In some cases, the support is a solid support or a stationary phase. Disclosed is that the support includes a bead, a particle, a nanoparticle or a microsphere.

[0142] In some examples, the agent individually is selected from among an antibody fragment, a monovalent antibody fragment, a proteinaceous binding molecule with antibody-like binding properties, a molecule containing Ig domains, and binding fragments thereof.

[0143] In some examples, the agent, the receptor-binding agent, which can be the second receptor-binding agent, and/or the first receptor-binding agent contains an antibody fragment. Disclosed is that the agent, the receptor-binding agent, which can be the second receptor-binding agent, and/or the first receptor-binding agent contains a Fab fragment. In some cases, the agent, the receptor-binding agent, which can be the second receptor-binding agent, and/or the first receptor-binding agent is a divalent antibody fragment such as a $F(ab')_2$-fragment or a divalent single-chain Fv (scFv) fragment. In some examples, the agent, the receptor-binding agent, which can be the second receptor-binding agent, and/or the first receptor-binding agent is a monovalent antibody fragment selected from among a Fab fragment, an Fv fragment and an scFv fragment.

[0144] In some examples, the agent or first receptor-binding agent includes an agent that specifically binds to CD3. In some examples, the agent that specifically binds CD3 is an

anti-CD3-antibody, a divalent antibody fragment of an anti-CD3 antibody, a monovalent antibody fragment of an anti-CD3-antibody, or a proteinaceous CD3 binding molecule with antibody-like binding properties. In some examples, the agent or receptor-binding agent, which can be the second receptor-binding agent, includes an agent that specifically binds to CD90, CD95, CD137, CD154, ICOS, LAT, CD27, OX40 and HVEM, such as an anti-CD90-antibody, a divalent antibody fragment of an anti-CD90 antibody, an antibody fragment of an anti-CD90-antibody, a proteinaceous CD90 binding molecule with antibody-like binding properties, an anti-CD95-antibody, a divalent antibody fragment of an anti-CD95 antibody, an antibody fragment of an anti-CD95-antibody, a proteinaceous CD95 binding molecule with antibody-like binding properties, an anti-CD154-antibody, a divalent antibody fragment of an anti-CD154 antibody, a monovalent antibody fragment of an anti-CD154-antibody, a proteinaceous CD154 binding molecule with antibody-like binding properties, an anti-CD137-antibody, a divalent antibody fragment of an anti-CD137 antibody, a monovalent antibody fragment of an anti-CD137-antibody, a proteinaceous CD137 binding molecule with antibody-like binding properties, an anti-ICOS-antibody, a divalent antibody fragment of an anti-ICOS antibody, a monovalent antibody fragment of an anti-ICOS-antibody, a proteinaceous ICOS binding molecule with antibody-like binding properties, an anti-LAT-antibody, a divalent antibody fragment of an anti-LAT antibody, a monovalent antibody fragment of an anti-LAT-antibody, a proteinaceous LAT binding molecule with antibody-like binding properties, an anti-CD27-antibody, a divalent antibody fragment of an anti-CD27 antibody, a monovalent antibody fragment of an anti-CD27-antibody, a proteinaceous CD27 binding molecule with antibody-like binding properties, an anti-OX40-antibody, a divalent antibody fragment of an anti-OX40 antibody, a monovalent antibody fragment of an anti-OX40-antibody, a proteinaceous OX40 binding molecule with antibody-like binding properties, an anti-HVEM-antibody, a divalent antibody fragment of an anti-HVEM antibody, a monovalent antibody fragment of an anti-HVEM-antibody, a proteinaceous HVEM binding molecule with antibody-like binding properties, or any mixture thereof.

[0145] In some examples, the reagent is or contains streptavidin, avidin, a streptavidin analog or mutein or and an avidin analog or mutein. In some examples, the reagent includes an oligomer or polymer of streptavidin, avidin, a streptavidin analog or mutein or and an avidin analog or mutein.

[0146] Disclosed is a kit, including the reagent disclosed herein and optionally instructions for use. In some examples, the kit contains a substance capable of reversing the bond between the receptor-binding agent and the reagent. In some examples, the kit contains a reagent containing a plurality of binding sites capable of reversibly binding to a receptor-binding agent. Disclosed is that the receptor-binding agent is reversibly bound to the reagent and is capable of specifically binding to a molecule expressed on the surface of target cells. In some instances, binding to the molecule induces or modulates a signal in the target cells. In some cases the kit contains a substance capable of reversing the bond between the receptor-binding agent and the reagent.

[0147] In some examples, the target cells are T cells. In some examples, the reagent is or includes streptavidin, avidin, a streptavidin analog or mutein or and an avidin analog or mutein. In some examples, the reagent includes an oligomer or polymer of streptavidin, avidin, a streptavidin analog or mutein or and an avidin analog or mutein.

[0148] In some examples, the substance includes a streptavidin-binding peptide, biotin or a biologically active fragment, optionally a D-biotin, or a biotin analog or biologically active fragment.

[0149] In some examples, the reagent has a size that is less than 20 nm, less than 10 nm, less than 5 nm or less than 1 nm. In some examples, the reagent has a density of less than 1.2 g/cm3 or less than 1.0 g/cm3. In some examples, the reagent is not bound to a support or solid support.

[0150] Disclosed is a composition including a plurality of T cells genetically engineered to express a recombinant receptor that specifically binds to a target antigen. In some cases, greater than 35%, 40%, 50%, 60%, 70%, 80% or 90% of the cells include a T cell subset containing a surface phenotype that is CD3+, CD4+ or CD8+ and CD62L+ and one or more of CD127+, CD45RA+, CD45RO-, CCR7+ and CD27+ and one or more of t-bet^{low}, IL-7Ra+, CD95+, IL-2Rβ+, CXCR3+ and LFA-1+as a percentage of the total T cells in the composition or the total cells in the composition. In some examples, prior to or during the genetic engineering, the plurality of T cells containing the T cell subset were not incubated in the presence of a GSK-P inhibitor; were not incubated in the presence of a recombinant homeostatic cytokine, optionally IL-7 or IL-15; or were not enriched for CD62L+ cells. In some examples, the composition does not contain a GSK-P inhibitor or a recombinant homeostatic cytokine, optionally IL-7 or IL-15.

[0151] In some examples, the T cell subset includes at least 5 X 10^6, at least 1 x 10^6 or at least 2 x 10^6 cells.

[0152] Provided herein in some examplesis a composition including a plurality of T cells genetically engineered to express a recombinant receptor that specifically binds to a target antigen. Disclosed is that the genetically engineered T cells are derived from transducing a population of T cells containing a T cell subset containing a surface phenotype that is CD3+, CD4+ or CD8+ and CD62L+ and one or more of CD127+, CD45RA+, CD45RO-, CCR7+ and CD27+ and one or more of t-bet^{low}, IL-7Ra+, CD95+, IL-2Rβ+, CXCR3+ and LFA-1+. In some examples, the T cell subset is present at a greater percentage of the total T cells in the population or a greater number of total T cells in the population compared to a population containing primary T cells that were isolated or enriched from a human subject based on surface expression of one or markers containing the phenotype. In some examples, the T cell subset is present at a greater percentage of the total T cells in the population or a greater number of total T cells in the population compared to a population of T cells that were incubated in the presence of a GSK-P inhibitor. In some examples, the T cell subset is present at a greater percentage of the total T cells in the population or a greater number of total T cells in the population compared to a population of T cells that were incubated in the presence of a recombinant homeostatic cytokine, optionally IL-7 or IL-15. In some examples, the T cell subset is present at a greater percentage of the total T cells in the population or a greater number of total T cells in the population compared to a population of T cells that were stimulated by anti-CD3 and anti-CD8, but in which the stimulation or activation was for greater than 1 day, 2 days, 3 days, 4 days or 5 days and/or the stimulation was not disrupted in the presence of biotin or a biotin analog.

[0153] In some examples, the T cell subset is present in the population at or about greater than 35%, 40%, 50%, 60%, 70%, 80% or 90% as a percentage of the total T cells in the population. In some examples, the T cell subset includes at least 5 x 10^6 cells, 1 x10^6 cells, 2 x10^6 cells or more.

[0154] In some examples, the composition is a pharmaceutical composition.

[0155] Disclosed is a method of treatment including administering to a subject having a disease or condition a composition, *e.g.*, pharmaceutical composition, as described herein.

[0156] In some examples, the cells include a recombinant receptor, such as a chimeric antigen receptor (CAR) or TCR. Disclosed is that the recombinant receptor, such as CAR or transgenic TCR specifically binds to an antigen associated with the disease or condition.

[0157] In some examples, the disease or condition is a cancer, and autoimmune disease or disorder, or an infectious disease.

Brief Description of the Drawings

[0158] **FIG. 1(A-E)** provides schematic representations of exemplary embodiments.

[0159] **FIG. 1A** shows a schematic representation of a reagent (or representative portion thereof) with a plurality of binding sites for reversible binding to agents. In this case, the reagent is shown as capable of reversibly binding to two agents, each of which is capable of specifically binding to a molecule on a cell. The reagent has a plurality of binding sites, including a plurality of the binding site, Z1, each capable of reversibly binding to the agents. The first and second agents, which, in some cases, can be the same, in the schematic representation shown each contain at least one binding partner C1. Binding partner C1 reversibly binds to binding site Z1. The first and second agents each also contain a binding site, B2, which can specifically bind to a molecule on the surface of a cell, which, in some cases, can be on the same cell. Here, the first and second agents are shown specifically binding to molecules on the same cell.

[0160] **FIG. 1B** shows a schematic representation of a reagent with a plurality of binding sites, capable of reversibly binding to a first and second agent, which agents are each capable of specifically binding to a molecule on a first and second cell, respectively. The reagent has a plurality of binding sites Z1, each capable of reversibly binding to an agent. The first and second agents, which, in some cases, can be the same, each contain a binding partner C1, which reversibly binds to binding site Z1. The first and second agents each contain a binding site B2, which can specifically bind to a molecule on the surface of a cell, which, in some cases, can be on the same cell or a different cell. Here, the first agent is bound to a molecule on the surface of a first cell and the second agent is bound to a molecule on the surface of a second cell.

[0161] **FIG. 1C** shows a reagent capable of reversibly binding to a first and second agents, which agents are each capable of specifically binding to a molecule on a first and second cell, respectively. The reagent has a plurality of binding sites Z1 and Z2, which can be the same or different, each capable of reversibly binding to one or both of the agents. The first agent contains a binding partner C1, which reversibly binds to Z1; the second agent contains a binding

CLAIMS

WHAT IS CLAIMED:

1. A method for culturing T cells, the method comprising:

(a) incubating a composition comprising T cells in the presence of a receptor-binding agent, wherein the receptor-binding agent i) is reversibly bound to a reagent comprising a plurality of binding sites capable of reversibly binding to the receptor-binding agent and ii) specifically binds to a molecule on the surface of the T cells in a manner that induces or modulates a signal in T cells in the composition; and

(b) within 5 days after initiation of said incubation, disrupting the reversible binding between the receptor-binding agent and the reagent, thereby generating cultured T cells.

2. The method of claim 1, wherein:

binding of the receptor-binding agent to the molecule initiates a TCR/CD3 complex-associated signal in the T cells; and/or

the receptor-binding agent specifically binds to a member of a TCR/CD3 complex; and/or

the receptor-binding agent specifically binds to CD3.

3. The method of claim 1 or claim 2, wherein the molecule is a component of the TCR/CD3 complex or is CD3.

4. The method of any of claims 1-3, wherein said disruption comprises introducing to the cells a composition comprising a substance capable of reversing the bond between the receptor-binding agent and the reagent.

5. The method of any of claims 1-4, wherein the receptor-binding agent is a first receptor-binding agent and the incubation is further carried out in the presence of a second receptor-binding agent, which is capable of specifically binding to a second molecule on the surface of one or more of the T cells.

6. The method of claim 5, wherein the binding of the second receptor-binding agent to the second molecule enhances, dampens, or modifies a signal delivered through the first molecule in the T cells.

7. The method of claim 5 or claim 6, wherein the reagent comprises a plurality of binding sites capable of reversibly binding to the second receptor-binding agent, whereby the second receptor-binding agent is reversibly bound to the reagent.

8. The method of claim 5 or claim 6, wherein the reagent is a first reagent and the incubation is carried out in the presence of at least a second reagent which is reversibly bound to the second receptor-binding agent.

9. The method of any of claims 5-8, wherein said disruption comprises introducing to the cells a composition comprising a substance capable of reversing the bond between the first receptor-binding agent and the reagent and/or the second receptor-binding agent and the reagent.

10. A method for culturing T cells, the method comprising:

(a) incubating a composition comprising T cells in the presence of:

i) a first receptor-binding agent that specifically binds to a first molecule expressed on the surface of the T cells in a manner that induces or modulates a TCR/CD3 complex-associated signal in the T cell; and

ii) a second receptor-binding agent, wherein the second receptor-binding agent i) is reversibly bound to a reagent comprising a plurality of binding sites capable of reversibly binding to the second receptor-binding agent and ii) specifically binds to a second molecule on the surface of the T cells in a manner to induce or modulate a second signal in the T cells; and

(b) within 5 days after initiation of said incubation, disrupting the reversible binding between the second receptor-binding agent and the reagent, thereby generating cultured T cells.

11. The method of claim 10, wherein the second signal enhances, dampens or modifies a signal delivered through the first molecule in the T cells.

12. The method of claim 10 or claim 11, wherein said disruption is carried out greater than 30 minutes after initiation of said incubation.

13. The method of any of claims 10-12, wherein:

said disruption is carried out between 1 hour and 4 days after initiation of said incubation, between 6 hours and 3 days after initiation of said incubation, between 12 hours and 2 days after initiation of said incubation or between 1 day and 3 days after initiation of said incubation; or

said disruption is carried out between about 1 hour and about 4 days after initiation of said incubation, between about 6 hours and about 3 days after initiation of said incubation, between about 12 hours and about 2 days after initiation of said incubation or between about 1 day and about 3 days after initiation of said incubation; or

said disruption is carried out greater than or equal to about 1 hour after initiation of said incubation and within 1 day, 2 days, 3 days or 4 days after initiation of said incubation.

14. The method of any of claims 5-13, wherein:

the second signal is a signal other than a TCR/CD3 complex-associated signal;

the second signal is capable of enhancing or potentiating a TCR/CD3 complex-associated signal; or

the second molecule is a costimulatory molecule, accessory molecule, cytokine receptor, chemokine receptor, immune checkpoint molecule or is a member of the TNF family or the TNF receptor family.

15. The method of any of claims 5-14, wherein the second molecule is selected from among CD28, CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40 and HVEM.

16. The method of any of claims 5-15, wherein the second molecule is CD28.

17. The method of any of claims 1-16, comprising, after said disruption, further incubating the composition comprising the T cells.

18. The method of claim 17, wherein:

the incubation and further incubation are carried out in the same vessel; and/or

the further incubation is carried out in the presence of the substance; and/or

the method does not comprise removing the substance, receptor-binding agent, second receptor-binding agent and/or reagent from the cell composition prior to the further incubation; and/or

the incubation and/or further incubation is carried out at or about 37 °C ± 2 °C; and/or

the incubation and/or further incubation is carried out in the presence of a further agent that is capable of delivering a signal to T cells.

19. The method of claim 17 or claim 18, wherein the incubation or further incubation is carried out in the presence of a further agent capable of enhancing or inducing proliferation of T cells, CD4+ T cells and/or CD8+ T cells.

20. The method of claim 19, wherein the further agent is a cytokine selected from among IL-2, IL-15 and IL-7.

21. The method of any of claims 17-20, wherein the further incubation is carried out for a time that is no more than 14 days, no more than 12 days, no more than 10 days, no more than 8 days or no more than 6 days.

22. The method of any of claims 1-20, wherein the T cells are primary cells from a subject.

23. The method of any of claims 1-22, wherein the T cells are unfractionated T cells, are enriched or isolated CD3+ T cells, are enriched or isolated CD4+ T cells or are enriched or isolated CD8+ T cells.

24. The method of any of claims 1-23, wherein the T cells are human cells.

25. The method of any of claims 1-24, wherein:

the reagent is not, and is not bound to or associated with, a solid support, stationary phase, a bead, a microparticle, a magnetic particle, and/or a matrix during said incubation; and/or

the reagent is flexible, does not contain a metal or magnetic core, is comprised entirely or primarily of organic multimer, is not spherical, is not substantially spherical or uniform in shape and/or is not rigid.

26. The method of any of claims 1-25, wherein the reagent is bound to a support during at least a portion of the incubation, whereby a plurality of the T cells or target cells are reversibly immobilized on the support during at least a portion of the incubation.

27. The method of any of claims 1-26, wherein the each receptor-binding agent individually is selected from among an antibody fragment, a monovalent antibody fragment, a proteinaceous binding molecule with antibody-like binding properties, a molecule containing Ig domains, a cytokine, a chemokine, an aptamer, and MHC molecule and binding fragments thereof.

28. The method of any of claims 1-27, wherein:

the receptor-binding agent comprise an antibody fragment;

the receptor-binding agent comprise a Fab fragment;

the receptor-binding agent is a divalent antibody fragment selected from among a $F(ab')_2$-fragment and a divalent single-chain Fv (scFv) fragment;

the receptor-binding agent is a monovalent antibody fragment selected from among a Fab fragment, an Fv fragment and an scFv fragment; and/or

the receptor-binding agent is a proteinaceous binding molecule with antibody-like binding properties selected from among aptamers, muteins based on a polypeptide of the lipocalin family, glubodies, proteins based on the ankyrin scaffold, proteins based on the crystalline scaffold, adnectins and avimers.

29. The method of any of claims 1-28, wherein the reagent is or comprises streptavidin, avidin, mutein of streptavidin that reversibly binds biotin, a biotin analog or a biologically active fragment thereof; mutein of avidin or streptavidin that reversibly binds a

streptavidin-binding peptide; a reagent that comprises at least two chelating groups K, wherein the at least two chelating groups are capable of binding to a transition metal ion; an agent capable of binding to an oligohistidine affinity tag; an agent capable of binding to a glutathione-S-transferase; calmodulin or an analog thereof; an agent capable of binding to calmodulin binding peptide (CBP); an agent capable of binding to a FLAG-peptide; an agent capable of binding to an HA-tag; an agent capable of binding to maltose binding protein (MBP); an agent capable of binding to an HSV epitope; an agent capable of binding to a myc epitope; or an agent capable of binding to a biotinylated carrier protein.

30. The method of any of claims 1-29, wherein the reagent is an oligomer or polymer of streptavidin, avidin, a mutein of streptavidin that reversibly binds biotin or a biologically active fragment; a streptavidin or avidin mutein that reversibly binds a streptavidin-binding peptide; a reagent that comprises at least two chelating groups K, wherein the at least two chelating groups are capable of binding to a transition metal ion; an agent capable of binding to an oligohistidine affinity tag; an agent capable of binding to a glutathione-S-transferase; calmodulin or an analog thereof; an agent capable of binding to calmodulin binding peptide (CBP); an agent capable of binding to a FLAG-peptide; an agent capable of binding to an HA-tag; an agent capable of binding to maltose binding protein (MBP); an agent capable of binding to an HSV epitope; an agent capable of binding to a myc epitope; or an agent capable of binding to a biotinylated carrier protein.

31. The method of any of claims 1-30, wherein the reagent comprises an oligomer or polymer of a streptavidin mutein.

32. The method of claim 30 or 31, wherein individual molecules of the oligomer or polymer are crosslinked by a polysaccharide or a bifunctional linker.

33. The method of any of claims 1-32, wherein:

the reagent comprises a streptavidin mutein comprising the amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1;or

the streptavidin analog or mutein comprises the amino acid sequence Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} at sequence positions corresponding to positions 44 to 47 with reference to positions in streptavidin in the sequence of amino acids set forth in SEQ ID NO:1.

34. The method of any of claims 1-33, wherein the streptavidin mutein comprises:

a) the sequence of amino acids set forth in any of SEQ ID NOS: 3-6, 27 and 28;

b) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS:3-6, 27 and 28 and contains the amino acid sequence corresponding to Val^{44}-Thr^{45}-Ala^{46}-Arg^{47} or Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} and that reversibly binds to biotin or a biologically active form thereof, a biotin analog or mutein or a biologically active fragment thereof or a streptavidin-binding peptide; or

c) a functional fragment of a) or b) that reversibly binds to biotin or a biologically active form thereof, a biotin analog or mutein or a biologically active fragment thereof or a streptavidin-binding peptide.

35. The method of any of claims 29-34, wherein:

the reagent is or comprises a streptavidin mutein ; and

the streptavidin-binding peptide is selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

36. The method of any of claims 1-35, wherein said disruption comprises introducing to the cells a composition comprising a substance capable of reversing the bond between the receptor-binding agent and the reagent.

37. The method of claim 36, wherein the substance is a free binding partner and/or is a competition agent.

38. The method of any of claims 1-37, wherein:

the reagent is or comprises a streptavidin analog or mutein or biologically active fragments thereof; and

the substance comprises a streptavidin-binding peptide, biotin or a biologically active fragment, optionally a D-biotin, or a biotin analog or biologically active fragment.

39. The method of claim 38, wherein:

the substance is a streptavidin-binding peptide is selected from the group consisting of Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 8), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_3$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 17), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 18) and Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(GlyGlyGlySer)_2$Gly-Gly-Ser-Ala-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO: 19).

40. The method of any of claims 1-39, wherein:

prior to the incubation, contacting cells with a selection agent that specifically binds to a marker comprised by T cells or target cells of the composition, thereby generating or obtaining the composition comprising the T cells or target cells; or

at least a portion of the incubation is carried out further in the presence of a selection agent that specifically binds to a marker comprised by T cells or target cells of the composition, wherein the cultured T cells are enriched for T cells or target cells comprising the marker.

41. The method of claim 40, wherein:

the selection agent is reversibly bound to the reagent, said reagent further comprising a plurality of binding sites capable of specifically binding the selection agent; or

the selection agent is reversibly bound to a further reagent comprising a plurality of binding sites capable of specifically binding to the selection agent.

42. The method of any of claims 1-41, wherein the induction or modulation of the signal effects an increase in expansion and/or activation of the cultured T cells compared to incubation of T cells in the absence of the induction or modulation of the signal.

43. The method of any of claims 1-42 wherein the method increases expansion and/or proliferation of T cells in the composition, alters the metabolic profile of T cells in the composition, alters the subset of CD8+ T cells in the composition; and/or increases the percentage of long-lived memory T cells in the composition, each compared to the T cells in the composition prior to the incubation, subsequent to the incubation but in the absence of the disruption or subsequent to the incubation but in the presence of an agent that specifically binds CD28 and/or induces or modulates CD28 signaling.

44. The method of any of claims 1-43, further comprising introducing a recombinant nucleic acid molecule into T cells of the population, which nucleic acid molecule encodes a recombinant protein, whereby cells express the recombinant protein.

45. The method of claim 44, wherein the recombinant receptor is a chimeric antigen receptor or transgenic T cell receptor (TCR).