has been shown that human $CD39^{hi}$ regulatory T cells exhibits stronger stability, higher Foxp3 expression, and suppressive ability under inflammatory conditions (Gu et al., *Cell Mol Immunol* 14(6): 521-528, 2017). Alternations in Treg cell development, homeostasis or function can predispose these cells to a variety of disease conditions including allergy, autoimmunity, graft rejection, cancer, and response to immunotherapies (Sakaguchi et al., *Annu Rev Immunol* 38: 541-566, 2020). Current research is focused on developing novel therapies to enhance Treg cell functions in vivo through use of cytokines and small molecular weight drugs to support endogenous Treg cell proliferation or activation, ex-vivo manipulated Treg cells in autologous adoptive cell therapy (ACT) to promote immunoregulation in settings of autoimmunity, or antigen-specific Treg cells, including chimeric antigen receptor Treg (CAR-Treg) cells, to strengthen tolerance in allergic inflammation (Ferreira et al., *Nat Rev Drug Discov* 18(10): 749-769, 2019). The present disclosure is a method that uses Treg cells to deactivate the inflammasome and then subsequently uses immune cells activated by one or more immunotherapeutics to reduce the accumulated senescent cells in an individual to treat inflamm-aging and/or any aging-associated pathologies. Provided is a method of inhibiting inflammasome related diseases and inhibiting senescent related diseases. The Treg cells can be in-vivo enhanced endogenous Treg cells or ex-vivo manipulated Treg cells used in an ACT setting. The immune cells for senescent-cell clearance could be activated by immunotherapeutics in vivo or generated by ex-vivo stimulation and expansion methods to support an ACT administration. The present disclosure is based on the discovery that single-chain chimeric polypeptides that include a soluble tissue factor domain or multi-chain chimeric polypeptides that include a soluble tissue factor domain, in combination with CD3/CD28-binding agents or IL-2 receptor-activating agents are effective in stimulating or increasing the proliferation of a T_{reg} cell. Based on this discovery provided herein are methods of stimulating or increasing the proliferation of a T_{reg} cell that include culturing a T_{reg} cell in a liquid culture medium over a period of time, where at the beginning of the period of time, the liquid culture medium includes: a CD3/CD28-binding agent; and a single-chain chimeric polypeptide including a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, where the

first target-binding domain and the second target-binding domain bind to a receptor for IL-2.

In a **first aspect**, the present disclosure refers to a method of stimulating or increasing the proliferation of a T_{reg} cell, wherein the method comprises:

culturing a T_{reg} cell in a liquid culture medium over a period of time, wherein at the beginning of the period of time, the liquid culture medium comprises:

a CD3/CD28-binding agent; and

a single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein the first target-binding domain and the second target-binding domain bind to a receptor for IL-2.

In a **second aspect**, the present disclosure refers to a population of T_{reg} cells generated by any one of the methods of the first aspect.

In a **third aspect**, the present disclosure refers to a composition comprising the population of T_{reg} cells of the second aspect and a pharmaceutically acceptable carrier.

In a **fourth aspect**, the present disclosure refers to use of the composition of the third aspect in the manufacture of a medicament for treating a subject in need thereof.

In a **fifth aspect**, the present disclosure refers to a kit comprising:

(i) a CD3/CD28-binding agent;

(ii) a single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein the first target-binding domain and the second target-binding domain bind to a receptor for IL-2; and

(iii) an mTOR inhibitor.

In a **sixth aspect**, the present disclosure refers to method of stimulating or increasing the proliferation of a T_{reg} cell, wherein the method comprises:

culturing a T_{reg} cell in a liquid culture medium over a period of time, wherein at the beginning of the period of time, the liquid culture medium comprises:

an IL-2 receptor-activating agent; and

a single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein: the first target-binding domain of the additional single-chain chimeric polypeptide binds to CD3 and the second target-binding domain of the additional single-chain chimeric polypeptide binds to CD28.

In a **seventh aspect**, the present disclosure refers to a population of T_{reg} cells generated by any one of the methods of the sixth aspect.

In an **eighth aspect**, the present disclosure refers to a composition comprising the population of T_{reg} cells of the seventh aspect and a pharmaceutically acceptable carrier.

In a **ninth aspect**, the present disclosure refers to use of the composition of the eighth aspect in the manufacture of a medicament for treating a subject in need thereof.

In a **tenth aspect**, the present disclosure refers to a kit comprising:

(i) an interleukin-2 receptor-activating agent;

(ii) a single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein:

the first target-binding domain binds to CD3 and the second target-binding domain binds to CD28; and

(iii) an mTOR inhibitor.

In an **eleventh aspect**, the present disclosure refers to a method of stimulating or increasing the proliferation of a T_{reg} cell, wherein the method comprises:

culturing a T_{reg} cell in a liquid culture medium over a period of time, wherein at the beginning of the period of time, the liquid culture medium comprises:

a CD3/CD28-binding agent; and

a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains; and

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains.

In a **twelfth aspect**, the present disclosure refers to population of T_{reg} cells generated by any one of the methods of the eleventh aspect.

In a **thirteenth aspect**, the present disclosure refers to a composition comprising the population of T_{reg} cells of the twelfth aspect and a pharmaceutically acceptable carrier.

In a **fourteenth aspect**, the present disclosure refers to use of the composition of the thirteenth aspect in the manufacture of a medicament for treating a subject in need thereof.

In a **fifteenth aspect**, the present disclosure refers to a kit comprising:

a CD3/CD28-binding agent;

a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

an mTOR inhibitor.

In a **sixteenth aspect**, the present disclosure refers to a method of isolating a T_{reg} cell from a sample obtained from a subject, wherein the method comprises separating the T_{reg} cell from the sample based on its expression of CD39, thereby isolating the T_{reg} cell.

In a **seventeenth aspect**, the present disclosure refers to a population of isolated T_{reg} cells generated by the method of the sixteenth aspect.

In an **eighteenth aspect**, the present disclosure refers to method of expanding a population of isolated T_{reg} cells of the seventeenth aspect, comprising culturing the population of isolated T_{reg} cells under conditions that allow for proliferation of the isolated T_{reg} cells.

In a **ninteenth aspect**, the present disclosure refers to a composition comprising the population of isolated T_{reg} cells of seventeenth aspect and a pharmaceutically acceptable carrier.

In a **twentieth aspect**, the present disclosure refers to use of the composition of nineteenth aspect in the manufacture of a medicament for treating a subject in need thereof.

In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the single-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the IgG1 antibody construct comprises a heavy chain variable domain comprising CDRs of SEQ ID NOs: 66, 67 or 74, and 68,

and a light chain variable domain comprising CDRs of SEQ ID NOs: 69, 70, and 71. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the heavy chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 72, and the light chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 73. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain and the soluble tissue factor domain directly abut each other. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain and the second target-binding domain directly abut each other. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain and the second target-binding domain comprise the same amino acid sequence. In some examples of any of the

methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain and the second target-binding domain comprise a soluble IL-2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble IL-2 is a human soluble IL-2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain and the second target-binding domain each comprise a sequence that is at least 80% identical to SEQ ID NO: 1. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain and the second target-binding domain each comprise a sequence that is at least 90% identical to SEQ ID NO: 1. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain and the second target-binding domain each comprise a sequence of SEQ ID NO: 1. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain is a soluble human tissue factor domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain comprises a sequence of SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain does not comprise any of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid

position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain comprises one or more of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain does not initiate blood coagulation. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide does not initiate blood coagulation. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 3. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 3. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence

of SEQ ID NO: 3. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 4. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 4. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 4. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 10 nM to about 500 nM of the single-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 50 nM to about 150 nM of the single-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the CD3/CD28-binding agent is an anti-CD3/anti-CD28 bead. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises the anti-CD3/anti-CD28 bead at a ratio of about 1:1 to about 6:1 beads/cell. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises the anti-CD3/anti-CD28 bead at a ratio of about 3:1 to about 5:1 beads/cell. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the CD3/CD28-binding agent is an additional single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein: the first target-binding domain of the additional single-chain chimeric polypeptide binds to CD3 and the second target-binding domain of the additional single-chain chimeric polypeptide binds to CD28. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the additional single-chain chimeric polypeptide. In some examples of any of the

methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the IgG1 antibody construct comprises a heavy chain variable domain comprising CDRs of SEQ ID NOs: 66, 67 or 74, and 68, and a light chain variable domain comprising CDRs of SEQ ID NOs: 69, 70, and 71. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the heavy chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 72, and the light chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 73. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain of the additional single-chain chimeric polypeptide and the soluble tissue factor domain directly abut each other. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the additional single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain and the second target-binding domain of the additional single-chain chimeric polypeptide directly abut each other. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the additional single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the CD3 is human CD3. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain of the additional single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 5. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain of the additional single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 5. In some

examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain of the additional single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 5. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the CD28 is human CD28. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the second target-binding domain of the additional single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the second target-binding domain of the additional single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the second target-binding domain of the additional single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain of the additional single-chain chimeric polypeptide is a soluble human tissue factor domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain of the additional single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain of the additional single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain of the additional single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain of the single-chain chimeric polypeptide comprises or consists of a sequence from a wildtype soluble human tissue factor. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain does not

comprise any of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain comprises one or more of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain of the additional single-chain chimeric polypeptide does not initiate blood coagulation. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the additional single-chain chimeric polypeptide does not initiate blood coagulation. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the additional single-chain chimeric polypeptide comprises a sequence that is at least 80%

identical to SEQ ID NO: 7. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the additional single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 7. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the additional single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 7. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the additional single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 8. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the additional single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 8. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the additional single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 8. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 10 nM to about 1000 nM of the additional single-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 50 nM to about 300 nM of the additional single-chain chimeric polypeptide.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the CD3/CD28 binding agent is a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and wherein the first target-binding domain binds to CD3 and the second target-binding domain binds to CD28, or the first target-binding domain binds to CD28 and the second target-binding domain to CD3.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the multi-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the IgG1 antibody construct comprises a heavy chain variable domain comprising CDRs of SEQ ID NOs: 66, 67 or 74, and 68, and a light chain variable domain comprising CDRs of SEQ ID NOs: 69, 70, and 71. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the heavy chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 72, and the light chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 73. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first target-binding domain of the first chimeric polypeptide and the soluble tissue factor domain directly abut each other. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble tissue factor domain and the second target-binding domain of the second chimeric polypeptide directly abut each other. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the CD3 is human CD3. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first target-binding domain of the first chimeric

polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 5. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first target-binding domain of the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 5. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first target-binding domain of the first chimeric polypeptide comprises a sequence of SEQ ID NO: 5.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble tissue factor domain of the first chimeric polypeptide is a soluble human tissue factor domain. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble human tissue factor domain of the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble human tissue factor domain of the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble human tissue factor domain of the first chimeric polypeptide comprises a sequence of SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble tissue factor domain of the first chimeric polypeptide comprises or consists of a sequence from a wildtype soluble human tissue factor.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble tissue factor domain of the first chimeric polypeptide does not comprise any of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that

corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble tissue factor domain of the first chimeric polypeptide comprises one or more of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble tissue factor domain of the first chimeric polypeptide does not initiate blood coagulation. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 102. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 102. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first chimeric polypeptide comprises a sequence of SEQ ID NO: 102. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 104. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first

chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 104. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first chimeric polypeptide comprises a sequence of SEQ ID NO: 104.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the CD28 is human CD28. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second target-binding domain of the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second target-binding domain of the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second target-binding domain of the second chimeric polypeptide comprises a sequence of SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 106. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 106. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second chimeric polypeptide comprises a sequence of SEQ ID NO: 106. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 108. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 108. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second chimeric polypeptide comprises a sequence of SEQ ID NO: 108.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the multi-chain chimeric polypeptide does not initiate blood coagulation. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the liquid culture medium comprises about 10 nM to about 1000 nM of the multi-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the liquid culture medium comprises about 50 nM to about 300 nM of the multi-chain chimeric polypeptide.

In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium further comprises an mTOR inhibitor. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the mTOR inhibitor is rapamycin. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 10 nM to about 500 nM of the mTOR inhibitor. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 50 nM to about 150 nM of the mTOR inhibitor. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the liquid culture medium does not comprise an mTOR inhibitor.

In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the methods further include: periodically adding to the liquid culture medium, after each 36 hours to about 60 hours after the start of the period of time, the single-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the periodic addition of the single-chain chimeric polypeptide is performed to result in a concentration of about 10 nM to about 500 nM of the single-chain chimeric polypeptide in the liquid culture medium. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the periodic addition of the single-chain chimeric polypeptide is performed to result in a concentration of about 50 nM to about 150 nM of the single-chain chimeric polypeptide in the liquid

culture medium. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the methods further include: periodically adding to the liquid culture medium, after each about 6 days to about 8 days after the start of the period of time, the CD3/CD28-binding agent. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the period of time is about 7 days to about 56 days. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the period of claim is about 15 days to about 25 days. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the method further comprises, before the culturing step, a step of isolating the T_{reg} cell from a sample obtained from a subject. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the method further comprises, after the culturing step, a step of isolating the Treg cell from a sample obtained from a subject.

In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the isolating comprises the use of fluorescence-assorted cell sorting. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the T_{reg} cell is an autologous T_{reg} cell, a haploidentical T_{reg} cell, or an allogeneic T_{reg} cell isolated from peripheral blood or umbilical cord blood. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the T_{reg} cell is a $CD4^+CD25^+Foxp3^+$ cell. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the T_{reg} cell is a $CD4^+CD25^+CD127^{dim}$cell. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the T_{reg} cells comprises a chimeric antigen receptor.

In another example, provided herein are populations of T_{reg} cells generated by any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein. Provided herein are compositions comprising any of the population of T_{reg} cells generated by any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein and a pharmaceutically acceptable carrier. Also provided herein are

methods of treating a subject in need thereof, the methods include administering to the subject a therapeutically effective amount of any of the compositions comprising any of the population of T_{reg} cells generated by any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein and a pharmaceutically acceptable carrier. In some examples of any of the methods of treating a subject in need thereof, the subject has been identified or diagnosed as having an aging-related disease or an inflammatory disease. In some examples of any of the methods of treating a subject in need thereof, the aging-related disease is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, neurodegeneration, stroke, dementia, vascular disease, infection susceptibility, chronic inflammation, and renal dysfunction. In some examples of any of the methods of treating a subject in need thereof, the inflammatory disease is selected from the group consisting of: rheumatoid arthritis, inflammatory bowel disease, lupus erythematosus, lupus nephritis, diabetic nephropathy, CNS injury, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Crohn's disease, multiple sclerosis, Guillain-Barre syndrome, psoriasis, Grave's disease, ulcerative colitis, nonalcoholic steatohepatitis, and mood disorders.

In another example, provided herein are kits that include: (i) a CD3/CD28-binding agent; (ii) a single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein the first target-binding domain and the second target-binding domain bind to a receptor for IL-2; and (iii) an mTOR inhibitor. In some examples of any of the kits described herein, the kits further include an IgG1 antibody construct that comprises at least one antigen-

binding domain that binds specifically to the soluble tissue factor domain of the single-chain chimeric polypeptide. In some examples of any of the kits described herein, the CD3/CD28-binding agent is an anti-CD3/anti-CD28 bead. In some examples of any of the kits described herein, the CD3/CD28-binding agent is an additional single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein: the first target-binding domain of the additional single-chain chimeric polypeptide binds to CD3 and the second target-binding domain of the additional single-chain chimeric polypeptide binds to CD28. In some examples of any of the kits described herein, the kits further include an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the additional single-chain chimeric polypeptide.

In some examples of any of the kits described herein, the CD3/CD28-binding agent is a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, wherein: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and wherein the first target-binding domain binds to CD3 and the second target-binding domain binds to CD28, or the first target-binding domain binds to CD28 and the second target-binding domain to CD3. In some examples of any of the kits described herein, the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the multi-chain chimeric polypeptide.

In another example, provided herein are methods of stimulating or increasing the proliferation of a T_{reg} cell, the methods include: culturing a T_{reg} cell in a liquid culture medium over a period of time, wherein at the beginning of the period of time, the liquid culture medium comprises: an IL-2 receptor-activating agent; and a single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein: the first target-binding domain of the additional

single-chain chimeric polypeptide binds to CD3 and the second target-binding domain of the additional single-chain chimeric polypeptide binds to CD28. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the single-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the IgG1 antibody construct comprises a heavy chain variable domain comprising CDRs of SEQ ID NOs: 66, 67 or 74, and 68, and a light chain variable domain comprising CDRs of SEQ ID NOs: 69, 70, and 71. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the heavy chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 72, and the light chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 73. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain and the soluble tissue factor domain directly abut each other. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain and the second target-binding domain directly abut each other. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the CD3 is human CD3. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain comprises

a sequence that is at least 80% identical to SEQ ID NO: 5. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain comprises a sequence that is at least 90% identical to SEQ ID NO: 5. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain comprises a sequence of SEQ ID NO: 5. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the CD28 is human CD28. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the second target-binding domain comprises a sequence that is at least 80% identical to SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the second target-binding domain comprises a sequence that is at least 90% identical to SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the second target-binding domain comprises a sequence of SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain is a soluble human tissue factor domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain comprises a sequence of SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain does not comprise any of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino

acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain comprises one or more of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain does not initiate blood coagulation. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide does not initiate blood coagulation. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 7. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 7. In

some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 7. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 8. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 8. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 8. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 10 nM to about 1000 nM of the single-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 50 nM to about 300 nM of the single-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the IL-2 receptor-activating agent is a soluble IL-2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble IL-2 is a human soluble IL-2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the human soluble IL-2 comprises a sequence that is at least 80% identical to SEQ ID NO: 1. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the human soluble IL-2 comprises a sequence that is at least 90% identical to SEQ ID NO: 1. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the human soluble IL-2 comprises a sequence of SEQ ID NO: 1. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 100 IU/mL to about 800 IU/mL of the human soluble IL-2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 400 IU/mL to about 600

IU/mL of the human soluble IL-2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium further comprises an mTOR inhibitor. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the mTOR inhibitor is rapamycin. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 10 nM to about 500 nM of the mTOR inhibitor. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 50 nM to about 150 nM of the mTOR inhibitor. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the liquid culture medium does not comprise an mTOR inhibitor.

In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the methods further include: periodically adding to the liquid culture medium, after each 36 hours to about 60 hours after the start of the period of time, the IL-2 receptor-activating agent. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the periodic addition of the IL-2 receptor-activating agent is performed to result in a concentration of about 10 nM to about 1000 nM of the IL-2 receptor-activating agent in the liquid culture medium. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the periodic addition of the IL-2 receptor activating agent is performed to result in a concentration of about 50 nM to about 150 nM of the IL-2 receptor-activating agent in the liquid culture medium. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the methods further include: periodically adding to the liquid culture medium, after each about 6 days to about 8 days after the start of the period of time, the single-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the period of time is about 7 days to about 56 days. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the period of claim is about 15 days to about 25 days. In some examples of any of the methods of

stimulating or increasing the proliferation of a T_{reg} cell described herein, the methods further include, before the culturing step, a step of isolating the T_{reg} cell from a sample obtained from a subject. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the method further comprises, after the culturing step, a step of isolating the Treg cell from a sample obtained from a subject.

In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the isolating comprises the use of fluorescence-assorted cell sorting. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the T_{reg} cell is an autologous T_{reg} cell, a haploidentical T_{reg} cell, or an allogeneic T_{reg} cell isolated from peripheral blood or umbilical cord blood. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the T_{reg} cell is a $CD4^+CD25^+Foxp3^+$ cell. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the T_{reg} cell is a $CD4^+CD25^+CD127^{dim}$ cell. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the T_{reg} cells comprises a chimeric antigen receptor.

Provided herein are populations of T_{reg} cells generated by any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein. Provided herein are compositions comprising any populations of T_{reg} cells generated by any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein and a pharmaceutically acceptable carrier. Also provided herein are methods of treating a subject in need thereof, the methods include administering to the subject a therapeutically effective amount of any of the compositions comprising any populations of T_{reg} cells generated by any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein and a pharmaceutically acceptable carrier. In some examples of any of the methods of treating a subject in need thereof described herein, the subject has been identified or diagnosed as having an aging-related disease or an inflammatory disease. In some examples of any of the methods of treating a subject in need thereof described herein, the aging-related disease is selected from the group consisting of:

Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, neurodegeneration, stroke, dementia, vascular disease, infection susceptibility, chronic inflammation, and renal dysfunction. In some examples of any of the methods of treating a subject in need thereof described herein, the inflammatory disease is selected from the group consisting of: rheumatoid arthritis, inflammatory bowel disease, lupus erythematosus, lupus nephritis, diabetic nephropathy, CNS injury, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Crohn's disease, multiple sclerosis, Guillain-Barre syndrome, psoriasis, Grave's disease, ulcerative colitis, nonalcoholic steatohepatitis, and mood disorders.

In another example, provided herein are kits that include: (i) an interleukin-2 receptor-activating agent; (ii) a single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein: the first target-binding domain binds to CD3 and the second target-binding domain binds to CD28; and (iii) an mTOR inhibitor. In some examples of any of the kits described herein, the kits further include an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the single-chain chimeric polypeptide.

In another example, provided herein are methods of stimulating or increasing the proliferation of a T_{reg} cell, the methods include: culturing a T_{reg} cell in a liquid culture medium over a period of time, wherein at the beginning of the period of time, the liquid culture medium comprises: a CD3/CD28-binding agent; and a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a first domain of a pair of

affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the multi-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the IgG1 antibody construct comprises a heavy chain variable domain comprising CDRs of SEQ ID NOs: 66, 67 or 74, and 68, and a light chain variable domain comprising CDRs of SEQ ID NOs: 69, 70, and 71. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the heavy chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 72, and the light chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 73. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the

first chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain and the second target-binding domain bind specifically to different antigens. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, one or both of the first target-binding domain and the second target-binding domain bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM-1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a

receptor for CD155, a receptor for CD122, and a receptor for CD28. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble interleukin, cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, and FLT3L. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble receptor is a soluble TGF-β receptor II (TGF-βRII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM-1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, or a soluble CD28. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first chimeric polypeptide further comprises one or more additional target-binding domain(s). In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the second chimeric polypeptide further comprises one or more additional target-binding domains. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL15Rα) and a soluble IL-15. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble IL-15 has a D8N or D8A amino acid substitution. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described

herein, the soluble tissue factor domain is a soluble human tissue factor domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain comprises a sequence of SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain does not comprise any of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain comprises one or more of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid

position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain does not initiate blood coagulation. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the multi-chain chimeric polypeptide does not initiate blood coagulation. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the CD3/CD28-binding agent is an anti-CD3/anti-CD28 bead. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises the anti-CD3/anti-CD28 bead at a ratio of about 1:1 to about 6:1 beads/cell. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises the anti-CD3/anti-CD28 bead at a ratio of about 3:1 to about 5:1 beads/cell. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the CD3/CD28-binding agent is an single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein: the first target-binding domain of the single-chain chimeric polypeptide binds to CD3 and the second target-binding domain of the single-chain chimeric polypeptide binds to CD28. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the single-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the IgG1 antibody construct comprises a heavy chain variable domain comprising CDRs of SEQ ID NOs: 66, 67 or 74, and 68, and a light chain variable domain comprising CDRs of SEQ ID NOs: 69, 70, and 71. In some

examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the heavy chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 72, and the light chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 73. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain of the single-chain chimeric polypeptide and the soluble tissue factor domain directly abut each other. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain and the second target-binding domain of the single-chain chimeric polypeptide directly abut each other. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the CD3 is human CD3. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain of the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 5. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain of the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 5. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the first target-binding domain of the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 5. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the CD28 is human CD28.

In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the second target-binding domain of the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the second target-binding domain of the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the second target-binding domain of the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain of the single-chain chimeric polypeptide is a soluble human tissue factor domain. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain of the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain of the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble human tissue factor domain of the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain of the single-chain chimeric polypeptide comprises or consists of a sequence from a wildtype soluble human tissue factor. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain does not comprise any of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino

acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain comprises one or more of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the soluble tissue factor domain of the single-chain chimeric polypeptide does not initiate blood coagulation. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide does not initiate blood coagulation. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 7. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 7. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 7. In some examples of any of the methods of

stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 8. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 8. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 8. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 10 nM to about 1000 nM of the single-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 50 nM to about 300 nM of the single-chain chimeric polypeptide.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the CD3/CD28 binding agent is an additional multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and wherein the first target-binding domain binds to CD3 and the second target-binding domain binds to CD28, or the first target-binding domain binds to CD28 and the second target-binding domain to CD3.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the additional multi-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the IgG1 antibody construct comprises a heavy chain variable domain comprising CDRs of SEQ ID NOs: 66, 67 or 74, and 68,

and a light chain variable domain comprising CDRs of SEQ ID NOs: 69, 70, and 71. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the heavy chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 72, and the light chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 73. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first target-binding domain of the first chimeric polypeptide and the soluble tissue factor domain of the additional multi-chain chimeric polypeptide directly abut each other. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first chimeric polypeptide of the additional multi-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble tissue factor domain and the second target-binding domain of the second chimeric polypeptide of the additional multi-chain chimeric polypeptide directly abut each other. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second chimeric polypeptide of the additional multi-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the CD3 is human CD3. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first target-binding domain of the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 5. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first target-binding domain of the additional single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO:

5. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first target-binding domain of the additional single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 5.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble tissue factor domain of the first chimeric polypeptide of the additional multi-chain chimeric polypeptide is a soluble human tissue factor domain. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble human tissue factor domain of the first chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble human tissue factor domain of the first chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble human tissue factor domain of the first chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 2. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble tissue factor domain of the first chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises or consists of a sequence from a wildtype soluble human tissue factor.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble tissue factor domain of the first chimeric polypeptide of the additional multi-chain chimeric polypeptide does not comprise any of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid

position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble tissue factor domain of the first chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises one or more of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the soluble tissue factor domain of the first chimeric polypeptide of the additional multi-chain chimeric polypeptide does not initiate blood coagulation.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 102. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 102. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first chimeric polypeptide of the additional multi-chain chimeric polypeptide

comprises a sequence of SEQ ID NO: 102. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 104. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first chimeric polypeptide of the additional multi-chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 104. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the first chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 104.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the CD28 is human CD28. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second target-binding domain of the second chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second target-binding domain of the second chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 6. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second target-binding domain of the second chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 6.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 106. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 106. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described

herein, the second chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 106. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 108. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 108. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the second chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 108.

In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the additional multi-chain chimeric polypeptide does not initiate blood coagulation. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the liquid culture medium comprises about 10 nM to about 1000 nM of the additional multi-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the liquid culture medium comprises about 50 nM to about 300 nM of the additional multi-chain chimeric polypeptide.

In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium further comprises an mTOR inhibitor. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the mTOR inhibitor is rapamycin. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 10 nM to about 500 nM of the mTOR inhibitor. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the liquid culture medium comprises about 50 nM to about 150 nM of the mTOR inhibitor. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the methods further include: periodically adding to the

liquid culture medium, after each 36 hours to about 60 hours after the start of the period of time, the multi-chain chimeric polypeptide. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the periodic addition of the multi-chain chimeric polypeptide is performed to result in a concentration of about 10 nM to about 500 nM of the multi-chain chimeric polypeptide in the liquid culture medium. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the periodic addition of the multi-chain chimeric polypeptide is performed to result in a concentration of about 50 nM to about 150 nM of the multi-chain chimeric polypeptide in the liquid culture medium. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the methods further include: periodically adding to the liquid culture medium, after each about 6 days to about 8 days after the start of the period of time, the CD3/CD28-binding agent. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the period of time is about 7 days to about 56 days. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the period of claim is about 15 days to about 25 days. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the method further comprises, before the culturing step, a step of isolating the T_{reg} cell from a sample obtained from a subject. In some examples of any of the methods of stimulating or increasing the proliferation of a Treg cell described herein, the method further comprises, after the culturing step, a step of isolating the Treg cell from a sample obtained from a subject.

In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the isolating comprises the use of fluorescence-assorted cell sorting. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the T_{reg} cell is an autologous T_{reg} cell, a haploidentical T_{reg} cell, or an allogeneic T_{reg} cell isolated from peripheral blood or umbilical cord blood. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the T_{reg} cell is a $CD4^+CD25^+Foxp3^+$ cell. In some examples of any of the methods of stimulating or

increasing the proliferation of a T_{reg} cell described herein, the T_{reg} cell is a $CD4^+CD25^+CD127^{dim}$ cell. In some examples of any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein, the T_{reg} cells comprises a chimeric antigen receptor.

Provided herein are populations of T_{reg} cells generated by any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein. Also provided herein are compositions comprising any of the populations of T_{reg} cells generated by any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein and a pharmaceutically acceptable carrier. Provided herein are methods of treating a subject in need thereof, the methods include administering to the subject a therapeutically effective amount of any of the compositions comprising any of the populations of T_{reg} cells generated by any of the methods of stimulating or increasing the proliferation of a T_{reg} cell described herein and a pharmaceutically acceptable carrier. In some examples of any of the methods of treating a subject in need thereof described herein, the subject has been identified or diagnosed as having an aging-related disease or an inflammatory disease. In some examples of any of the methods of treating a subject in need thereof described herein, the aging-related disease is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, neurodegeneration, stroke, dementia, vascular disease, infection susceptibility, chronic inflammation, and renal dysfunction. In some examples of any of the methods of treating a subject in need thereof described herein, the inflammatory disease is selected from the group consisting of: rheumatoid arthritis, inflammatory bowel disease, lupus erythematosus, lupus nephritis, diabetic nephropathy, CNS injury,

Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Crohn's disease, multiple sclerosis, Guillain-Barre syndrome, psoriasis, Grave's disease, ulcerative colitis, nonalcoholic steatohepatitis, and mood disorders.

In another example, provided herein are kits that include: a CD3/CD28-binding agent; a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and an mTOR inhibitor. In some examples of any of the kits described herein, the kits further include an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the multi-chain chimeric polypeptide. In some examples of any of the kits described herein, the CD3/CD28-binding agent is an anti-CD3/anti-CD28 bead. In some examples of any of the kits described herein, the CD3/CD28-binding agent is an single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein the first target-binding domain of the additional single-chain chimeric polypeptide binds to CD3 and the second target-binding domain of the additional single-chain chimeric polypeptide binds to CD28. In some examples of any of the kits described herein, the kits further include an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the single-chain chimeric polypeptide.

In some examples of any of the kits described herein, the CD3/CD28-binding agent is an additional multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and wherein the first target-binding domain binds

to CD3 and the second target-binding domain binds to CD28, or the first target-binding domain binds to CD28 and the second target-binding domain to CD3.

In some examples of any of the kits described herein, the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the additional multi-chain chimeric polypeptide.

In another example, provided herein are methods of isolating a Treg cell from a sample obtained from a subject, wherein the method comprises separating the Treg cell from the sample based on its expression of CD39, thereby isolating the Treg cell.

In some examples of any of the methods of isolating a Treg cell described herein, the method comprises: mixing the sample with an antibody or ligand capable of binding CD39 under conditions that allow binding of the antibody or ligand to Treg cells expressing CD39; separating the Treg cell bound to the antibody or ligand from other components in the sample, thereby isolating the Treg cell.

In some examples of any of the methods of isolating a Treg cell described herein, the antibody is a mouse, a humanized, or a human antibody or antigen-binding fragment thereof; and/or the antibody or the ligand is labeled with at least one of biotin, avidin, streptavidin, or a fluorochrome, or is bound to a particle, bead, resin, or solid support. In some examples of any of the methods of isolating a Treg cell described herein, separating comprises the use of flow cytometry, fluorescence-activated cell sorting (FACS), centrifugation, or column, plate, particle, or bead-based methods.

In some examples of any of the methods of isolating a Treg cell described herein, the method further comprises: mixing the sample with a biotinylated antibody or ligand capable of binding CD39 under conditions that allow binding of the antibody or the ligand to the Treg cell; capturing the Treg cell bound to the biotinylated antibody or the ligand using streptavidin-coated magnetic particles; separating the magnetic particle-bound Treg cell using a magnet; washing the magnet particle-bound Treg cell to remove other components in the sample; and releasing the magnetic particle-bound Treg cell from the magnet into a solution, thereby isolating the Treg cell.

In some examples of any of the methods of isolating a Treg cell described herein, the Treg cell is an autologous Treg cell, a haploidentical Treg cell, or an

allogeneic Treg cell isolated from a sample comprising fresh or frozen peripheral blood, umbilical cord blood, peripheral blood mononuclear cells, lymphocytes, $CD4^+$ T cells or Treg cells. In some examples of any of the methods of isolating a Treg cell described herein, the Treg cell is a $CD4^+CD25^+Foxp3^+$ cell. In some examples of any of the methods of isolating a Treg cell described herein, the Treg cell is a $CD4^+CD25^+CD127^{dim}$ cell. In some examples of any of the methods of isolating a Treg cell described herein, the Treg cells comprises a chimeric antigen receptor. In some examples of any of the methods of isolating a Treg cell described herein, the Treg cells are immunosuppressive in vitro and in vivo.

In another example, provided herein are populations of isolated Treg cells generated by any one of the methods described herein. In some examples of any of the populations of isolated Treg cells described herein, the isolated population of Treg cells are greater than 70% $CD39^+$ cells. Also provided are method of expanding any of the populations of isolated Treg cells described herein that include culturing the population of isolated Treg cells under conditions that allow for proliferation of the isolated Treg cells.

Also provided herein are compositions comprising any of the populations of isolated Treg cells described herein and a pharmaceutically acceptable carrier.

Also provided herein are methods of treating a subject in need thereof, comprising administering to the subject a therapeutically effective amount of any of the compositions described herein. In some examples of any of the methods of treating a subject in need described herein, the subject has been identified or diagnosed as having an aging-related disease or an inflammatory disease. In some examples of any of the methods of treating a subject in need described herein, the aging-related disease is selected from the group consisting of: Alzheimer's disease, aneurysm, cystic fibrosis, fibrosis in pancreatitis, glaucoma, hypertension, idiopathic pulmonary fibrosis, inflammatory bowel disease, intervertebral disc degeneration, macular degeneration, osteoarthritis, type 2 diabetes mellitus, adipose atrophy, lipodystrophy, atherosclerosis, cataracts, COPD, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, myocardial infarction, sarcopenia, wound healing, alopecia, cardiomyocyte hypertrophy, osteoarthritis, Parkinson's disease, age-associated loss of lung tissue elasticity, macular

degeneration, cachexia, glomerulosclerosis, liver cirrhosis, NAFLD, osteoporosis, amyotrophic lateral sclerosis, Huntington's disease, spinocerebellar ataxia, multiple sclerosis, neurodegeneration, stroke, dementia, vascular disease, infection susceptibility, chronic inflammation, and renal dysfunction. In some examples of any of the methods of treating a subject in need described herein, the inflammatory disease is selected from the group consisting of: rheumatoid arthritis, inflammatory bowel disease, lupus erythematosus, lupus nephritis, diabetic nephropathy, CNS injury, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Crohn's disease, multiple sclerosis, Guillain-Barre syndrome, psoriasis, Grave's disease, ulcerative colitis, nonalcoholic steatohepatitis, and mood disorders.

As used herein, the term "chimeric" refers to a polypeptide that includes amino acid sequences (e.g., domains) originally derived from two different sources (e.g., two different naturally-occurring proteins, e.g., from the same or different species). For example, a chimeric polypeptide can include domains from at least two different naturally occurring human proteins. In some examples, a chimeric polypeptide can include a domain that is a synthetic sequence (e.g., a scFv) and a domain that is derived from a naturally-occurring protein (e.g., a naturally-occurring human protein). In some examples, a chimeric polypeptide can include at least two different domains that are synthetic sequences (e.g., two different scFvs).

An "antigen-binding domain" is one or more protein domain(s) (e.g., formed from amino acids from a single polypeptide or formed from amino acids from two or more polypeptides (e.g., the same or different polypeptides) that is capable of specifically binding to one or more different antigen(s). In some examples, an antigen-binding domain can bind to an antigen or epitope with specificity and affinity similar to that of naturally-occurring antibodies. In some examples, the antigen-binding domain can be an antibody or a fragment thereof. In some examples, an antigen-binding domain can include an alternative scaffold. Non-limiting examples of antigen-binding domains are described herein. Additional examples of antigen-binding domains are known in the art.

A "soluble tissue factor domain" refers to a polypeptide having at least 70% identity (e.g., at least 75% identity, at least 80% identity, at least 85% identity, at least

characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter may be lost in the presence of denaturing solvents. An epitope may comprise amino acid residues that are directly involved in the binding, and other amino acid residues, which are not directly involved in the binding. Methods for identifying an epitope to which an antigen-binding domain binds are known in the art.

The term "treatment" means to ameliorate at least one symptom of a disorder. In some examples, the disorder being treated is cancer and to ameliorate at least one symptom of cancer includes reducing aberrant proliferation, gene expression, signaling, translation, and/or secretion of factors. Generally, the methods of treatment include administering a therapeutically effective amount of composition that reduces at least one symptom of a disorder to a subject who is in need of, or who has been determined to be in need of such treatment.

The term "CD3/CD28-binding agent" means an agent that binds specifically to both CD3 and CD28 on the surface of a mammalian cell (e.g., a T cell, such as a T_{reg} cell) and induces downstream signaling in the mammalian cell. Non-limiting examples of CD3/CD28-binding agents are described herein.

The term "IL-2 receptor activating agent" means an agent that binds specifically to an IL-2 receptor present on a surface of a mammalian cell (e.g., a T cell, such as a T_{reg} cell) and induces downstream signaling in the mammalian cell. Non-limiting examples of IL-2 receptor activating agents are described herein.

The term "IgG1 antibody construct" means a single chain polypeptide or a multi-chain polypeptide that includes at least one antigen-binding domain that binds to a soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and at least one IgG1 Fc domain. Non-limiting examples of IgG1 antibody constructs are described herein.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting.

All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the disclosure will be apparent from the following detailed description and figures, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a diagram showing expansion of Foxp3$^+$ T_{reg} cells using either (1) 2t2, CD3/CD28 beads, and rapamycin or (2) recombinant human IL-2, CD3/CD28 beads, and rapamycin.

FIGs. 2A-2I are diagrams showing an increase in Helios$^+$, CTLA-4$^+$, CD39$^+$, CD62L$^+$, CD25$^+$, CD103$^+$, CD69$^+$, PD1$^+$, and CD49b$^+$ markers after stimulation of T_{reg} cells with (1) 2t2, CD3/CD28 beads, and rapamycin or (2) recombinant human IL-2, CD3/CD28 beads, and rapamycin.

FIGs. 3A-3D are diagrams showing glucose metabolism levels upon activation of human T_{reg} cells with (1) 2t2, CD3/CD28 beads, and rapamycin, or (2) recombinant human IL-2, CD3/CD28 beads, and rapamycin. FIG. 3A shows levels of glycolysis of T_{reg} cells. FIG. 3B shows levels of glycolytic capacity of T_{reg} cells. FIG. 3C shows levels of glycolytic reserve of T_{reg} cells. FIG. 3D shows levels of non-glycolytic acidification of T_{reg} cells.

FIG. 4 is a diagram showing the suppression of autologous T responder cells by T_{reg} cells previously stimulated with (1) 2t2, CD3/CD28 beads, and rapamycin, or (2) recombinant human IL-2, CD3/CD28 beads, and rapamycin.

FIG. 5 is a diagram showing the levels of IL-10 secretion by T_{reg} cells in a T responder cell suppression assay, where the T_{reg} cells were previously stimulated with either (1) 2t2 and CD3/CD28 beads in the presence or absence of rapamycin, or (2) recombinant human IL-2 and CD3/CD28 beads in the presence or absence of rapamycin.

FIGs. 6A-6B show exemplary diagrams for a fusion protein generated including anti-CD3scFv/TF/IL15D8N mutant (3t15*) and anti-CD28scFv/IL15RαSu (28s) fusion proteins.

WHAT IS CLAIMED IS:

1. A method of stimulating or increasing the proliferation of a T_{reg} cell, wherein the method comprises:

culturing a T_{reg} cell in a liquid culture medium over a period of time, wherein at the beginning of the period of time, the liquid culture medium comprises:

a CD3/CD28-binding agent; and

a single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein the first target-binding domain and the second target-binding domain bind to a receptor for IL-2.

2. The method of claim 1, wherein the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the single-chain chimeric polypeptide.

3. The method of claim 1 or 2, wherein the soluble tissue factor domain does not initiate blood coagulation.

4. The method of any one of claims 1-3, wherein the single-chain chimeric polypeptide does not initiate blood coagulation.

5. The method of any one of claims 1-4, wherein the CD3/CD28-binding agent is an anti-CD3/anti-CD28 bead.

6. The method of any one of claims 1-4, wherein the CD3/CD28-binding agent is an additional single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein:
the first target-binding domain of the additional single-chain chimeric polypeptide binds to CD3 and the second target-binding domain of the additional single-chain chimeric polypeptide binds to CD28.

7. The method of claim 6, wherein the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the additional single-chain chimeric polypeptide.

8. The method of any one claims 1-4, wherein the CD3/CD28 binding agent is a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains; and

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

wherein the first target-binding domain binds to CD3 and the second target-binding domain binds to CD28, or the first target-binding domain binds to CD28 and the second target-binding domain to CD3.

9. The method of claim 8, wherein the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the multi-chain chimeric polypeptide.

10. The method of any one of claims 1-9, wherein the method further comprises, before the culturing step, a step of isolating the T_{reg} cell from a sample obtained from a subject.

11. The method of any one of claims 1-9, wherein the method further comprises, after the culturing step, a step of isolating the T_{reg} cell from a sample obtained from a subject.

12. The method of any one of claims 1-11, wherein the T_{reg} cells comprises a chimeric antigen receptor.

13. A population of T_{reg} cells generated by the method of any one of claims 1-12.

14. A composition comprising the population of T_{reg} cells of claim 13 and a pharmaceutically acceptable carrier.

15. Use of the composition of claim 14 in the manufacture of a medicament for treating a subject in need thereof.

16. A kit comprising:

(i) a CD3/CD28-binding agent;

(ii) a single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein the first target-binding domain and the second target-binding domain bind to a receptor for IL-2; and

(iii) an mTOR inhibitor.

17. A method of stimulating or increasing the proliferation of a T_{reg} cell, wherein the method comprises:

culturing a T_{reg} cell in a liquid culture medium over a period of time, wherein at the beginning of the period of time, the liquid culture medium comprises:

an IL-2 receptor-activating agent; and

a single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein: the first target-binding domain of the additional single-chain chimeric

polypeptide binds to CD3 and the second target-binding domain of the additional single-chain chimeric polypeptide binds to CD28.

18. The method of claim 17, wherein the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the single-chain chimeric polypeptide.

19. The method of claim 17 or 18, wherein the liquid culture medium further comprises an mTOR inhibitor.

20. The method of any one of claims 17-19, wherein the method further comprises, before the culturing step, a step of isolating the T_{reg} cell from a sample obtained from a subject.

21. The method of any one of claims 17-19, wherein the method further comprises, after the culturing step, a step of isolating the Treg cell from a sample obtained from a subject.

22. The method of any one of claims 17-21, wherein the T_{reg} cells comprises a chimeric antigen receptor.

23. A population of T_{reg} cells generated by the method of any one of claims 17-22.

24. A composition comprising the population of T_{reg} cells of claim 23 and a pharmaceutically acceptable carrier.

25. Use of the composition of claim 24 in the manufacture of a medicament for treating a subject in need thereof.

26. The use of claim 25, wherein the subject has been identified or diagnosed as having an aging-related disease or an inflammatory disease.

27. A kit comprising:

(i) an interleukin-2 receptor-activating agent;

(ii) a single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein:

the first target-binding domain binds to CD3 and the second target-binding domain binds to CD28; and

(iii) an mTOR inhibitor.

28. A method of stimulating or increasing the proliferation of a T_{reg} cell, wherein the method comprises:

culturing a T_{reg} cell in a liquid culture medium over a period of time, wherein at the beginning of the period of time, the liquid culture medium comprises:

a CD3/CD28-binding agent; and

a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains; and

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains.

29. The method of claim 28, wherein the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the multi-chain chimeric polypeptide.

30. The method of claim 28 or 29, wherein the CD3/CD28-binding agent is an anti-CD3/anti-CD28 bead.

31. The method of claim 28 or 29, wherein the CD3/CD28-binding agent is an single-chain chimeric polypeptide comprising a first target-binding domain, a soluble tissue factor domain, and a second target-binding domain, wherein:

the first target-binding domain of the single-chain chimeric polypeptide binds to CD3 and the second target-binding domain of the single-chain chimeric polypeptide binds to CD28.

32. The method of claim 31, wherein the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the single-chain chimeric polypeptide.

33. The method of claim 28 or 29, wherein the CD3/CD28 binding agent is an additional multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains; and

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

wherein the first target-binding domain binds to CD3 and the second target-binding domain binds to CD28, or the first target-binding domain binds to CD28 and the second target-binding domain to CD3.

34. The method of claim 33, wherein the liquid culture medium further includes an IgG1 antibody construct that comprises at least one antigen-binding domain that binds specifically to the soluble tissue factor domain of the additional multi-chain chimeric polypeptide.

35. The method of any one of claims 28-34, wherein the liquid culture medium further comprises an mTOR inhibitor.

36. The method of any one of claims 28-35, wherein the method further comprises, before the culturing step, a step of isolating the T_{reg} cell from a sample obtained from a subject.

37. The method of any one of claims 28-35, wherein the method further comprises, after the culturing step, a step of isolating the T_{reg} cell from a sample obtained from a subject.

38. The method of any one of claims 28-37, wherein the T_{reg} cells comprises a chimeric antigen receptor.

39. A population of T_{reg} cells generated by the method of any one of claims 28-38.

40. A composition comprising the population of T_{reg} cells of claim 39 and a pharmaceutically acceptable carrier.

41. Use of the composition of claim 40 in the manufacture of a medicament for treating a subject in need thereof.

42. The use of claim 41, wherein the subject has been identified or diagnosed as having an aging-related disease or an inflammatory disease.

43. A kit comprising:

a CD3/CD28-binding agent;

a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

an mTOR inhibitor.

44. A method of isolating a T_{reg} cell from a sample obtained from a subject, wherein the method comprises separating the T_{reg} cell from the sample based on its expression of CD39, thereby isolating the T_{reg} cell.

45. A population of isolated T_{reg} cells generated by the method of claim 44.

46. A method of expanding a population of isolated T_{reg} cells of claim 45, comprising culturing the population of isolated T_{reg} cells under conditions that allow for proliferation of the isolated T_{reg} cells.

47. A composition comprising the population of isolated T_{reg} cells of claim 45 and a pharmaceutically acceptable carrier.

48. Use of the composition of claim 47 in the manufacture of a medicament for treating a subject in need thereof.