

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 2, wherein 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.
4. The method of claim 2, wherein 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.
5. The method of claim 4, wherein the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73.
6. The method of any one of claims 1-5, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other.

7. The method of any one of claims 1-5, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain.

8. The method of any one of claims 1-7, wherein the soluble tissue factor domain and the second target-binding domain directly abut each other.

9. The method of any one of claims 1-7, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

10. The method of any one of claims 1-9, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

11. The method of any one of claims 1-10, wherein the first target-binding domain and the second target-binding domain comprise a soluble IL-2.

12. The method of claim 11, wherein the soluble IL-2 is a human soluble IL-2.

13. The method of any one of claims 1-12, wherein 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.

14. The method of claim 13, wherein 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.

15. The method of claim 14, wherein the first target-binding domain and the second target-binding domain each comprise a sequence of SEQ ID NO: 1.

16. The method of any one of claims 1-15, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

17. The method of claim 16, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 2.

18. The method of claim 17, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 2.

19. The method of claim 18, wherein the soluble human tissue factor domain comprises a sequence of SEQ ID NO: 2.

20. The method of any one of claims 1-15, wherein the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor.

21. The method of any one of claims 1-15, wherein 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.

22. The method of any one of claims 1-15, wherein 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.

23. The method of any one of claims 1-22, wherein the soluble tissue factor domain does not initiate blood coagulation.

24. The method of claim 1-23, wherein the single-chain chimeric polypeptide does not initiate blood coagulation.

25. The method of claim 1, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 3.

26. The method of claim 25, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 3.

27. The method of claim 26, wherein the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 3.

28. The method of claim 27, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 4.

29. The method of claim 28, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 4.

30. The method of claim 29, wherein the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 4.

31. The method of any one of claims 1-30, wherein the liquid culture medium comprises about 10 nM to about 500 nM of the single-chain chimeric polypeptide.

32. The method of claim 31, wherein the liquid culture medium comprises about 50 nM to about 150 nM of the single-chain chimeric polypeptide.

33. The method of any one of claims 1-32, wherein the CD3/CD28-binding agent is an anti-CD3/anti-CD28 bead.

34. The method of claim 33, wherein the liquid culture medium comprises the anti-CD3/anti-CD28 bead at a ratio of about 1:1 to about 6:1 beads/cell.

35. The method of claim 34, wherein the liquid culture medium comprises the anti-CD3/anti-CD28 bead at a ratio of about 3:1 to about 5:1 beads/cell.

36. The method of any one of claims 1-32, 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.

37. The method of claim 36, 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.

38. The method of claim 37, wherein 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.

39. The method of claim 38, wherein 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.

40. The method of claim 39, wherein the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73.

41. The method of any one of claims 36-40, wherein the first target-binding domain of the additional single-chain chimeric polypeptide and the soluble tissue factor domain directly abut each other.

42. The method of any one of claims 36-40, wherein the additional single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain.

43. The method of any one of claims 36-42, wherein the soluble tissue factor domain and the second target-binding domain of the additional single-chain chimeric polypeptide directly abut each other.

44. The method of any one of claims 36-42, wherein the additional single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

45. The method of any one of claims 36-44, wherein the CD3 is human CD3.

46. The method of claim 45, wherein 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.

47. The method of claim 46, wherein 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.

48. The method of claim 47, wherein the first target-binding domain of the additional single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 5.

49. The method of any one of claims 36-48, wherein the CD28 is human CD28.

50. The method of claim 49, wherein 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.

51. The method of claim 50, wherein 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.

52. The method of claim 51, wherein the second target-binding domain of the additional single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 6.

53. The method of any one of claims 36-52, wherein the soluble tissue factor domain of the additional single-chain chimeric polypeptide is a soluble human tissue factor domain.

54. The method of claim 53, wherein 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.

55. The method of claim 54, wherein 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.

56. The method of claim 55, wherein the soluble human tissue factor domain of the additional single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 2.

57. The method of any one of claims 36-52, wherein the soluble tissue factor domain of the single-chain chimeric polypeptide comprises or consists of a sequence from a wildtype soluble human tissue factor.

58. The method of any one of claims 36-52, wherein 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.

59. The method of any one of claims 36-52, wherein 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.

60. The method of any one of claims 36-59, wherein the soluble tissue factor domain of the additional single-chain chimeric polypeptide does not initiate blood coagulation.

61. The method of claim 36-60, wherein the additional single-chain chimeric polypeptide does not initiate blood coagulation.

62. The method of claim 36, wherein the additional single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 7.

63. The method of claim 62, wherein the additional single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 7.

64. The method of claim 63, wherein the additional single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 7.

65. The method of claim 64, wherein the additional single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 8.

66. The method of claim 65, wherein the additional single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 8.

67. The method of claim 66, wherein the additional single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 8.

68. The method of any one of claims 36-67, wherein the liquid culture medium comprises about 10 nM to about 1000 nM of the additional single-chain chimeric polypeptide.

69. The method of claim 68, wherein the liquid culture medium comprises about 50 nM to about 300 nM of the additional single-chain chimeric polypeptide.

70. The method of any one claims 1-32, 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.

71. The method of claim 70, 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.

72. The method of claim 71, wherein 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.

73. The method of claim 72, wherein 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.

74. The method of claim 73, wherein the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73.

75. The method of any one of claims 70-74, wherein the first target-binding domain of the first chimeric polypeptide and the soluble tissue factor domain directly abut each other.

76. The method of any one of claims 70-74, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain.

77. The method of any one of claims 70-76, wherein the soluble tissue factor domain and the second target-binding domain of the second chimeric polypeptide directly abut each other.

78. The method of any one of claims 70-76, wherein the second chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

79. The method of any one of claims 70-78, wherein the CD3 is human CD3.

80. The method of claim 79, wherein the first target-binding domain of the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 5.

81. The method of claim 80, wherein the first target-binding domain of the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 5.

82. The method of claim 81, wherein the first target-binding domain of the first chimeric polypeptide comprises a sequence of SEQ ID NO: 5.

83. The method of any one of claims 70-82, wherein the soluble tissue factor domain of the first chimeric polypeptide is a soluble human tissue factor domain.

84. The method of claim 83, wherein 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.

85. The method of claim 84, wherein 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.

86. The method of claim 85, wherein the soluble human tissue factor domain of the first chimeric polypeptide comprises a sequence of SEQ ID NO: 2.

87. The method of any one of claims 70-82, wherein the soluble tissue factor domain of the first chimeric polypeptide comprises or consists of a sequence from a wildtype soluble human tissue factor.

88. The method of any one of claims 70-82, wherein 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.

89. The method of any one of claims 70-82, wherein 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.

90. The method of any one of claims 70-89, wherein the soluble tissue factor domain of the first chimeric polypeptide does not initiate blood coagulation.

91. The method of claim 70, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 102.

92. The method of claim 91, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 102.

93. The method of claim 92, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 102.

94. The method of claim 70, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 104.

95. The method of claim 94, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 104.

96. The method of claim 95, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 104.

97. The method of any one of claims 70-96, wherein the CD28 is human CD28.

98. The method of claim 97, wherein the second target-binding domain of the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 6.

99. The method of claim 98, wherein the second target-binding domain of the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 6.

100. The method of claim 99, wherein the second target-binding domain of the second chimeric polypeptide comprises a sequence of SEQ ID NO: 6.

101. The method of claim 70, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 106.

102. The method of claim 101, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 106.

103. The method of claim 102, wherein the second chimeric polypeptide comprises a sequence of SEQ ID NO: 106.

104. The method of claim 70, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 108.

105. The method of claim 104, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 108.

106. The method of claim 105, wherein the second chimeric polypeptide comprises a sequence of SEQ ID NO: 108.

107. The method of claim 70-106, wherein the multi-chain chimeric polypeptide does not initiate blood coagulation.

108. The method of any one of claims 70-107, wherein the liquid culture medium comprises about 10 nM to about 1000 nM of the multi-chain chimeric polypeptide.

109. The method of claim 108, wherein the liquid culture medium comprises about 50 nM to about 300 nM of the multi-chain chimeric polypeptide.

110. The method of any one of claims 1-109, wherein the liquid culture medium further comprises an mTOR inhibitor.

111. The method of claim 110, wherein the mTOR inhibitor is rapamycin.

112. The method of claim 110 or 111, wherein the liquid culture medium comprises about 10 nM to about 500 nM of the mTOR inhibitor.

113. The method of claim 112, wherein the liquid culture medium comprises about 50 nM to about 150 nM of the mTOR inhibitor.

114. The method of any one of claims 1-109, wherein the liquid culture medium does not comprise an mTOR inhibitor.

115. The method of any one of claims 1-114, further comprising:
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.

116. The method of claim 115, wherein 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.

117. The method of claim 116, wherein 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.

118. The method of any one of claims 1-117, further comprising:
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.

119. The method of any one of claims 1-118, wherein the period of time is about 7 days to about 56 days.

120. The method of claim 119, wherein the period of time is about 15 days to about 25 days.

121. The method of any one of claims 1-120, wherein the method further comprises, before the culturing step, a step of isolating the T_{reg} cell from a sample obtained from a subject.

122. The method of any one of claims 1-120, wherein the method further comprises, after the culturing step, a step of isolating the T_{reg} cell from a sample obtained from a subject.

123. The method of claim 121 or 122, wherein the isolating comprises the use of fluorescence-assorted cell sorting.

124. The method of any one of claims 1-123, wherein 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.

125. The method of any one of claims 1-124, wherein the T_{reg} cell is a CD4⁺CD25⁺Foxp3⁺ cell.

126. The method of any one of claims 1-124, wherein the T_{reg} cell is a CD4⁺CD25⁺CD127^{dim} cell.

127. The method of any one of claims 1-126, wherein the T_{reg} cells comprises a chimeric antigen receptor.

128. A population of T_{reg} cells generated by the method of any one of claims 1-127.

129. A composition comprising the population of T_{reg} cells of claim 128 and a pharmaceutically acceptable carrier.

130. A method of treating a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the composition of claim 129.

131. The method of claim 130, wherein the subject has been identified or diagnosed as having an aging-related disease or an inflammatory disease.

132. The method of claim 131, wherein 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.

133. The method of claim 131, wherein 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.

134. 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.

135. The kit of claim 134, wherein the kit further comprises 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.

136. The kit of claim 134 or 135, wherein the CD3/CD28-binding agent is an anti-CD3/anti-CD28 bead.

137. The kit of claim 134 or 135, 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.

138. The kit of claim 137, wherein the kit further comprises 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.

139. The kit of claim 134 or 135, 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.

140. The method of claim 139, 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.

141. 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.

142. The method of claim 141, 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.

143. The method of claim 142, wherein 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.

144. The method of claim 143, wherein 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.

145. The method of claim 144, wherein the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73.

146. The method of any one of claims 141-145, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other.

147. The method of any one of claims 141-145, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain.

148. The method of any one of claims 141-147, wherein the soluble tissue factor domain and the second target-binding domain directly abut each other.

149. The method of any one of claims 141-147, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

150. The method of any one of claims 141-149, wherein the CD3 is human CD3.

151. The method of claim 150, wherein the first target-binding domain comprises a sequence that is at least 80% identical to SEQ ID NO: 5.

152. The method of claim 151, wherein the first target-binding domain comprises a sequence that is at least 90% identical to SEQ ID NO: 5.

153. The method of claim 152, wherein the first target-binding domain comprises a sequence of SEQ ID NO: 5.

154. The method of any one of claims 141-153, wherein the CD28 is human CD28.

155. The method of claim 154, wherein the second target-binding domain comprises a sequence that is at least 80% identical to SEQ ID NO: 6.

156. The method of claim 155, wherein the second target-binding domain comprises a sequence that is at least 90% identical to SEQ ID NO: 6.

157. The method of claim 156, wherein the second target-binding domain comprises a sequence of SEQ ID NO: 6.

158. The method of any one of claims 141-157, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

159. The method of claim 158, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 2.

160. The method of claim 159, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 2.

161. The method of claim 160, wherein the soluble human tissue factor domain comprises a sequence of SEQ ID NO: 2.

162. The method of any one of claims 141-157, wherein the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor.

163. The method of any one of claims 141-157, wherein 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.

164. The method of any one of claims 141-157, wherein 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.

165. The method of any one of claims 141-164, wherein the soluble tissue factor domain does not initiate blood coagulation.

166. The method of claim 141-165, wherein the single-chain chimeric polypeptide does not initiate blood coagulation.

167. The method of claim 141, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 7.

168. The method of claim 167, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 7.

169. The method of claim 168, wherein the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 7.

170. The method of claim 169, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 8.

171. The method of claim 170, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 8.

172. The method of claim 171, wherein the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 8.

173. The method of any one of claims 141-172, wherein the liquid culture medium comprises about 10 nM to about 1000 nM of the single-chain chimeric polypeptide.

174. The method of claim 173, wherein the liquid culture medium comprises about 50 nM to about 300 nM of the single-chain chimeric polypeptide.

175. The method of any one of claims 141-174, wherein the IL-2 receptor-activating agent is a soluble IL-2.

176. The method of claim 175, wherein the soluble IL-2 is a human soluble IL-2.

177. The method of claim 176, wherein the human soluble IL-2 comprises a sequence that is at least 80% identical to SEQ ID NO: 1.

178. The method of claim 177, wherein the human soluble IL-2 comprises a sequence that is at least 90% identical to SEQ ID NO: 1.

179. The method of claim 178, wherein the human soluble IL-2 comprises a sequence of SEQ ID NO: 1.

180. The method of claim 176-179, wherein the liquid culture medium comprises about 100 IU/mL to about 800 IU/mL of the human soluble IL-2.

181. The method of claim 180, wherein the liquid culture medium comprises about 400 IU/mL to about 600 IU/mL of the human soluble IL-2.

182. The method of any one of claims 141-181, wherein the liquid culture medium further comprises an mTOR inhibitor.

183. The method of claim 182, wherein the mTOR inhibitor is rapamycin.

184. The method of claim 182 or 183, wherein the liquid culture medium comprises about 10 nM to about 500 nM of the mTOR inhibitor.

185. The method of claim 184, wherein the liquid culture medium comprises about 50 nM to about 150 nM of the mTOR inhibitor.

186. The method of any one of claims 141-181, wherein the liquid culture medium does not comprise an mTOR inhibitor.

187. The method of any one of claims 141-186, further comprising:
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.

188. The method of claim 187, wherein 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.

189. The method of claim 188, wherein 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.

190. The method of any one of claims 141-189, further comprising:
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.

191. The method of any one of claims 141-190, wherein the period of time is about 7 days to about 56 days.

192. The method of claim 191, wherein the period of claim is about 15 days to about 25 days.

193. The method of any one of claims 141-192, wherein the method further comprises, before the culturing step, a step of isolating the T_{reg} cell from a sample obtained from a subject.

194. The method of any one of claims 141-192, wherein the method further comprises, after the culturing step, a step of isolating the Treg cell from a sample obtained from a subject.

195. The method of claim 193 or 194, wherein the isolating comprises the use of fluorescence-assorted cell sorting.

196. The method of any one of claims 141-195, wherein 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.

197. The method of any one of claims 141-196, wherein the T_{reg} cell is a CD4⁺CD25⁺Foxp3⁺ cell.

198. The method of any one of claims 141-196, wherein the T_{reg} cell is a CD4⁺CD25⁺CD127^{dim} cell.

199. The method of any one of claims 141-198, wherein the T_{reg} cells comprises a chimeric antigen receptor.

200. A population of T_{reg} cells generated by the method of any one of claims 141-199.

201. A composition comprising the population of T_{reg} cells of claim 200 and a pharmaceutically acceptable carrier.

202. A method of treating a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the composition of claim 201.

203. The method of claim 202, wherein the subject has been identified or diagnosed as having an aging-related disease or an inflammatory disease.

204. The method of claim 203, wherein 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.

205. The method of claim 203, wherein 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.

206. 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.

207. The kit of claim 206, wherein the kit further comprises 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.

208. 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.

209. The method of claim 208, 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.

210. The method of claim 209, wherein 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.

211. The method of claim 210, wherein 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.

212. The method of claim 211, wherein the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73.

213. The method of any one of claims 208-212, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

214. The method of any one of claims 208-212, wherein 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.

215. The method of any one of claims 208-214, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

216. The method of any one of claims 208-214, wherein 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.

217. The method of any one of claims 208-216, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

218. The method of any one of claims 208-216, wherein 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.

219. The method of any one of claims 208-218, wherein the first target-binding domain and the second target-binding domain bind specifically to the same antigen.

220. The method of any one of claims 208-218, wherein the first target-binding domain and the second target-binding domain bind specifically to different antigens.

221. The method of any one of claims 208-220, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

222. The method of any one of claims 208-221, wherein 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, TNF α , 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- β RII), a ligand of TGF- β RIII, 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.

223. The method of any one of claims 208-220, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein.

224. The method of claim 223, wherein 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.

225. The method of any one of claims 208-220, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor.

226. The method of claim 225, wherein the soluble receptor is a soluble TGF- β receptor II (TGF- β RII), a soluble TGF- β RIII, 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.

227. The method of any one of claims 208-226, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s).

228. The method of any one of claims 208-227, wherein the second chimeric polypeptide further comprises one or more additional target-binding domains.

229. The method of any one of claims 208-228, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15.

230. The method of claim 229, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

231. The method of any one of claims 208-228, wherein 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.

232. The method of any one of claims 208-231, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

233. The method of claim 232, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 2.

234. The method of claim 233, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 2.

235. The method of claim 234, wherein the soluble human tissue factor domain comprises a sequence of SEQ ID NO: 2.

236. The method of any one of claims 208-231, wherein the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor.

237. The method of any one of claims 208-231, wherein 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.

238. The method of any one of claims 208-231, wherein 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.

239. The method of any one of claims 208-238, wherein the soluble tissue factor domain does not initiate blood coagulation.

240. The method of claim 208-239, wherein the multi-chain chimeric polypeptide does not initiate blood coagulation.

241. The method of any one of claims 208-240, wherein the CD3/CD28-binding agent is an anti-CD3/anti-CD28 bead.

242. The method of claim 241, wherein the liquid culture medium comprises the anti-CD3/anti-CD28 bead at a ratio of about 1:1 to about 6:1 beads/cell.

243. The method of claim 242, wherein the liquid culture medium comprises the anti-CD3/anti-CD28 bead at a ratio of about 3:1 to about 5:1 beads/cell.

244. The method of any one of claims 208-240, wherein the CD3/CD28-binding agent is 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 single-chain chimeric polypeptide binds to CD3 and the second target-binding domain of the single-chain chimeric polypeptide binds to CD28.

245. The method of claim 244, 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.

246. The method of claim 245, wherein 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.

247. The method of claim 246, wherein 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.

248. The method of claim 247, wherein the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73.

249. The method of any one of claims 244-248, wherein the first target-binding domain of the single-chain chimeric polypeptide and the soluble tissue factor domain directly abut each other.

250. The method of any one of claims 244-248, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain.

251. The method of any one of claims 244-250, wherein the soluble tissue factor domain and the second target-binding domain of the single-chain chimeric polypeptide directly abut each other.

252. The method of any one of claims 244-250, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

253. The method of any one of claims 244-252, wherein the CD3 is human CD3.

254. The method of claim 253, wherein 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.

255. The method of claim 254, wherein 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.

256. The method of claim 255, wherein the first target-binding domain of the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 5.

257. The method of any one of claims 244-256, wherein the CD28 is human CD28.

258. The method of claim 257, wherein 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.

259. The method of claim 258, wherein 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.

260. The method of claim 259, wherein the second target-binding domain of the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 6.

261. The method of any one of claims 244-260, wherein the soluble tissue factor domain of the single-chain chimeric polypeptide is a soluble human tissue factor domain.

262. The method of claim 261, wherein 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.

263. The method of claim 262, wherein 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.

264. The method of claim 263, wherein the soluble human tissue factor domain of the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 2.

265. The method of any one of claims 244-260, wherein the soluble tissue factor domain of the single-chain chimeric polypeptide comprises or consists of a sequence from a wildtype soluble human tissue factor.

266. The method of any one of claims 244-260, wherein 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.

267. The method of any one of claims 244-260, wherein 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.

268. The method of any one of claims 244-267, wherein the soluble tissue factor domain of the single-chain chimeric polypeptide does not initiate blood coagulation.

269. The method of claim 244-268, wherein the single-chain chimeric polypeptide does not initiate blood coagulation.

270. The method of claim 244, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 7.

271. The method of claim 270, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 7.

272. The method of claim 271, wherein the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 7.

273. The method of claim 272, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 8.

274. The method of claim 273, wherein the single-chain chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 8.

275. The method of claim 274, wherein the single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 8.

276. The method of any one of claims 244-275, wherein the liquid culture medium comprises about 10 nM to about 1000 nM of the single-chain chimeric polypeptide.

277. The method of claim 276, wherein the liquid culture medium comprises about 50 nM to about 300 nM of the single-chain chimeric polypeptide.

278. The method of any one claims 208-240, 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.

279. The method of claim 278, 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.

280. The method of claim 279, wherein 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.

281. The method of claim 280, wherein 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.

282. The method of claim 281, wherein the heavy chain variable domain comprises SEQ ID NO: 72, and the light chain variable domain comprises SEQ ID NO: 73.

283. The method of any one of claims 278-282, wherein 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.

284. The method of any one of claims 278-282, wherein 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.

285. The method of any one of claims 278-284, wherein 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.

286. The method of any one of claims 278-284, wherein 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.

287. The method of any one of claims 278-286, wherein the CD3 is human CD3.

288. The method of claim 287, wherein the first target-binding domain of the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 5.

289. The method of claim 288, wherein 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.

290. The method of claim 289, wherein the first target-binding domain of the additional single-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 5.

291. The method of any one of claims 278-290, wherein the soluble tissue factor domain of the first chimeric polypeptide of the additional multi-chain chimeric polypeptide is a soluble human tissue factor domain.

292. The method of claim 291, wherein 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.

293. The method of claim 292, wherein 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.

294. The method of claim 293, wherein 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.

295. The method of any one of claims 278-290, wherein 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.

296. The method of any one of claims 278-290, wherein 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.

297. The method of any one of claims 278-290, wherein 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.

298. The method of any one of claims 278-297, wherein the soluble tissue factor domain of the first chimeric polypeptide of the additional multi-chain chimeric polypeptide does not initiate blood coagulation.

299. The method of claim 278, wherein 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.

300. The method of claim 299, wherein 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.

301. The method of claim 300, wherein the first chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 102.

302. The method of claim 278, wherein 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.

303. The method of claim 302, wherein the first chimeric polypeptide of the additional multi-chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 104.

304. The method of claim 303, wherein the first chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 104.

305. The method of any one of claims 278-304, wherein the CD28 is human CD28.

306. The method of claim 305, wherein 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.

307. The method of claim 306, wherein 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.

308. The method of claim 307, wherein 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.

309. The method of claim 278, wherein 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.

310. The method of claim 309, wherein 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.

311. The method of claim 310, wherein the second chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 106.

312. The method of claim 311, wherein 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.

313. The method of claim 312, wherein 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.

314. The method of claim 313, wherein the second chimeric polypeptide of the additional multi-chain chimeric polypeptide comprises a sequence of SEQ ID NO: 108.

315. The method of claim 278-314, wherein the additional multi-chain chimeric polypeptide does not initiate blood coagulation.

316. The method of any one of claims 278-315, wherein the liquid culture medium comprises about 10 nM to about 1000 nM of the additional multi-chain chimeric polypeptide.

317. The method of claim 316, wherein the liquid culture medium comprises about 50 nM to about 300 nM of the additional multi-chain chimeric polypeptide.

318. The method of any one of claims 208-317, wherein the liquid culture medium further comprises an mTOR inhibitor.

319. The method of claim 318, wherein the mTOR inhibitor is rapamycin.

320. The method of claim 318 or 319, wherein the liquid culture medium comprises about 10 nM to about 500 nM of the mTOR inhibitor.

321. The method of claim 320, wherein the liquid culture medium comprises about 50 nM to about 150 nM of the mTOR inhibitor.

322. The method of any one of claims 208-317, wherein the liquid culture medium does not comprise an mTOR inhibitor.

323. The method of any one of claims 208-322, further comprising:
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.

324. The method of claim 323, wherein 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.

325. The method of claim 324, wherein 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.

326. The method of any one of claims 208-325, further comprising:
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.

327. The method of any one of claims 208-326, wherein the period of time is about 7 days to about 56 days.

328. The method of claim 327, wherein the period of claim is about 15 days to about 25 days.

329. The method of any one of claims 208-328, wherein the method further comprises, before the culturing step, a step of isolating the T_{reg} cell from a sample obtained from a subject.

330. The method of any one of claims 208-328, wherein the method further comprises, after the culturing step, a step of isolating the T_{reg} cell from a sample obtained from a subject.

331. The method of claim 329 or 330, wherein the isolating comprises the use of fluorescence-assorted cell sorting.

332. The method of any one of claims 208-331, wherein 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.

333. The method of any one of claims 208-332, wherein the T_{reg} cell is a CD4⁺CD25⁺Foxp3⁺ cell.

334. The method of any one of claims 208-332, wherein the T_{reg} cell is a CD4⁺CD25⁺CD127^{dim} cell.

335. The method of any one of claims 208-334, wherein the T_{reg} cells comprises a chimeric antigen receptor.

336. A population of T_{reg} cells generated by the method of any one of claims 208-335.

337. A composition comprising the population of T_{reg} cells of claim 336 and a pharmaceutically acceptable carrier.

338. A method of treating a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the composition of claim 337.

339. The method of claim 338, wherein the subject has been identified or diagnosed as having an aging-related disease or an inflammatory disease.

340. The method of claim 339, wherein 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.

341. The method of claim 339, wherein 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.

342. 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.

343. The kit of claim 342, wherein the kit further comprises 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.

344. The kit of claim 342 or 343, wherein the CD3/CD28-binding agent is an anti-CD3/anti-CD28 bead.

345. The kit of claim 342 or 343, wherein the CD3/CD28-binding agent is 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.

346. The kit of claim 345, wherein the kit further comprises 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.

347. The kit of claim 342 or 343, 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.

348. The method of claim 347, 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.

349. 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.

350. The method of claim 349, wherein the method comprises: stimulating or increasing the proliferation of a T_{reg} cell by culturing the sample in a liquid culture medium before isolating the T_{reg} cell.

351. The method of claim 349 or 350, wherein 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 T_{reg} cells expressing CD39;
separating the T_{reg} cell bound to the antibody or ligand from other components in the sample, thereby isolating the T_{reg} cell.

352. The method of claim 351, wherein:
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.

353. The method of claim 351, wherein separating comprises the use of flow cytometry, fluorescence-activated cell sorting (FACS), centrifugation, or column, plate, particle, or bead-based methods.

354. The method of claim 349, wherein 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 T_{reg} cell;
capturing the T_{reg} cell bound to the biotinylated antibody or the ligand using streptavidin-coated magnetic particles;
separating the magnetic particle-bound T_{reg} cell using a magnet;
washing the magnet particle-bound T_{reg} cell to remove other components in the sample; and
releasing the magnetic particle-bound T_{reg} cell from the magnet into a solution, thereby isolating the T_{reg} cell.

355. The method of any one of claims 349-354, wherein the T_{reg} cell is an autologous T_{reg} cell, a haploidentical T_{reg} cell, or an allogeneic T_{reg} cell isolated from a sample comprising fresh or frozen peripheral blood, umbilical cord blood, peripheral blood mononuclear cells, lymphocytes, CD4⁺ T cells or T_{reg} cells.

356. The method of any one of claims 349-355, wherein the T_{reg} cell is a CD4⁺CD25⁺Foxp3⁺ cell.

357. The method of any one of claims 349-356, wherein the T_{reg} cell is a CD4⁺CD25⁺CD127^{dim} cell.

358. The method of any one of claims 349-357, wherein the T_{reg} cells comprises a chimeric antigen receptor.

359. The method of any one of claims 349-358, wherein the T_{reg} cells are immunosuppressive *in vitro* and *in vivo*.

360. A population of isolated T_{reg} cells generated by the method of any one of claims 349-359.

361. A method of expanding a population of isolated T_{reg} cells of claim 360, comprising culturing the population of isolated T_{reg} cells under conditions that allow for proliferation of the isolated T_{reg} cells.

362. The population of isolated T_{reg} cells of claim 360, wherein the isolated population of T_{reg} cells are greater than 70% CD39⁺ cells.

363. A composition comprising the population of isolated T_{reg} cells of claim 360 or 362 and a pharmaceutically acceptable carrier.

364. A method of treating a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the composition of claim 363.

365. The method of claim 364, wherein the subject has been identified or diagnosed as having an aging-related disease or an inflammatory disease.

366. The method of claim 365, wherein 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.

367. The method of claim 365, wherein 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.