

What is claimed is:

1. A polypeptide comprising a modified IL-2, wherein the modified IL-2 comprises a D84Y substitution, wherein the amino acid positions correspond to the amino acid positions in SEQ ID NO: 1.
2. The polypeptide of claim 1, wherein the modified IL-2 has reduced affinity for CD122 compared to wild-type IL-2.
3. The polypeptide of any one of claims 1-2, wherein the modified IL-2 comprises at least one substitution at at least one amino acid position selected from H16, L19, M23, N88, and E95.
4. The polypeptide of any one of claims 1-3, wherein the modified IL-2 comprises a substitution at amino acid position H16, optionally wherein the substitution is selected from H16A, H16N, H16V, and H16T.
- ~~5. The polypeptide of claim 4, wherein the substitution is selected from H16A, H16N, H16V, and H16T.~~
- ~~6.5. The polypeptide of any one of claims 1-54, wherein the modified IL-2 comprises:~~
 - ~~a) a substitution at amino acid position L19, optionally wherein the substitution is selected from L19A, L19P, L19Q, L19Y, L19N, L19S, L19T, and L19V;~~
 - ~~b) a substitution at amino acid position M23, optionally wherein the substitution is selected from M23A, M23G, M23S, M23T, M23V, M23D, M23E, M23I, M23K, M23L, M23N, M23Q, M23R, and M23Y;~~
 - ~~c) a substitution at amino acid position N88, optionally wherein the substitution is selected from N88T, N88A, and N88S; and/or~~
 - ~~d) a substitution at amino acid position E95, optionally wherein the substitution is selected from E95Q, E95G, E95T, E95V, E95P, E95H, E95N, and E95Y.~~
- ~~7. The polypeptide of claim 6, wherein the substitution is selected from L19A, L19P, L19Q, L19Y, L19N, L19S, L19T, L19V.~~
- ~~8. The polypeptide of any one of claims 1-7, wherein the modified IL-2 comprises a substitution at amino acid position M23.~~

9. —The polypeptide of claim 8, wherein the substitution is selected from M23A, M23G, M23S, M23T, M23V, M23D, M23E, M23I, M23K, M23L, M23N, M23Q, M23R, and M23Y.

10. —The polypeptide of any one of claims 1-9, wherein the modified IL-2 comprises a substitution at amino acid position N88.

11. —The polypeptide of claim 10, wherein the substitution is selected from N88T, N88A, and N88S.

12. —The polypeptide of any one of claims 1-11, wherein the modified IL-2 comprises a substitution at amino acid position E95.

13. —The polypeptide of claim 12, wherein the substitution is selected from E95Q, E95G, E95T, E95V, E95P, E95H, E95N, and E95Y.

14.6. The polypeptide of any one of claims 1-13.5, wherein the modified IL-2 comprises at least one substitution that reduces affinity for CD132 compared to wild-type IL-2.

15.7. The polypeptide of any one of claims 1-14.6, wherein the modified IL-2 comprises at least one substitution at at least one amino acid position selected from Q22, R120, T123, Q126, S127, I129, and S130.

16.8. The polypeptide of claim 15.7, wherein the modified IL-2 comprises: a substitution at amino acid position Q22.

a) a substitution at amino acid position Q22, optionally wherein the substitution is selected from Q22A, Q22D, Q22G, Q22H, Q22K, Q22N, Q22R, Q22S, Q22T, Q22V, and Q22Y;

b) a substitution at amino acid position R120, optionally wherein the substitution is selected from R120A, R120D, R120E, R120F, R120G, R120H, R120K, R120N, R120Q, R120S, R120V, and R120Y;

c) a substitution at amino acid position T123, optionally wherein the substitution is selected from T123D, T123E, T123H, T123K, T123N, T123Q, and T123R;

d) a substitution at amino acid position Q126, optionally wherein the substitution is selected from Q126N, Q126A, and Q126Y;

e) a substitution at amino acid position S127, optionally wherein the substitution is selected from S127D, S127E, S127H, S127K, S127N, S127P, and S127R;

- f) a substitution at amino acid position I129, optionally wherein the substitution is selected from I129A, I129H, I129R, and I129; and/or
- g) a substitution at amino acid position S130, optionally wherein the substitution is selected from S130E, S130K, S130N, S130P, S130Q, and S130R.
17. ~~—The polypeptide of claim 16, wherein the substitution is selected from Q22A, Q22D, Q22G, Q22H, Q22K, Q22N, Q22R, Q22S, Q22T, Q22V, and Q22Y.~~
18. ~~—The polypeptide of any one of claims 15–17, wherein the modified IL-2 comprises a substitution at amino acid position R120.~~
19. ~~—The polypeptide of claim 18, wherein the substitution is selected from R120A, R120D, R120E, R120F, R120G, R120H, R120K, R120N, R120Q, R120S, R120V, and R120Y.~~
20. ~~—The polypeptide of any one of claims 15–19, wherein the modified IL-2 comprises a substitution at amino acid position T123.~~
21. ~~—The polypeptide of claim 20, wherein the substitution is selected from T123D, T123E, T123H, T123K, T123N, T123Q, and T123R.~~
22. ~~—The polypeptide of any one of claims 15–21, wherein the modified IL-2 comprises a substitution at amino acid position Q126.~~
23. ~~—The polypeptide of claim 22, wherein the substitution is selected from Q126N, Q126A, and Q126Y.~~
24. ~~—The polypeptide of any one of claims 15–23, wherein the modified IL-2 comprises a substitution at amino acid position S127.~~
25. ~~—The polypeptide of claim 24, wherein the substitution is selected from S127D, S127E, S127H, S127K, S127N, S127P, and S127R.~~
26. ~~—The polypeptide of any one of claims 15–25, wherein the modified IL-2 comprises a substitution at amino acid position I129.~~
27. ~~—The polypeptide of claim 26, wherein the substitution is selected from I129A, I129H, I129R, and I129S.~~
28. ~~—The polypeptide of any one of claims 15–27, wherein the modified IL-2 comprises a substitution at amino acid position S130.~~
29. ~~—The polypeptide of claim 28, wherein the substitution is selected from S130E, S130K, S130N, S130P, S130Q, and S130R.~~

30. — A polypeptide comprising a modified IL-2, wherein the modified IL-2 comprises at least one substitution at at least one amino acid position selected from Q22, R120, T123, S127, and S130.

31. — The polypeptide of claim 30, wherein the modified IL-2 has reduced affinity for CD132 compared to wild-type IL-2.

32. — The polypeptide of any one of claims 30-31, wherein the modified IL-2 comprises a substitution at amino acid position Q22.

33. — The polypeptide of claim 32, wherein the substitution is selected from Q22A, Q22D, Q22E, Q22G, Q22H, Q22K, Q22N, Q22P, Q22R, Q22S, Q22T, Q22V, and Q22Y.

34. — The polypeptide of any one of claims 30-33, wherein the modified IL-2 comprises a substitution at amino acid position R120.

35. — The polypeptide of claim 34, wherein the substitution is selected from R120A, R120D, R120E, R120F, R120G, R120H, R120K, R120N, R120P, R120Q, R120S, R120V, and R120Y.

36. — The polypeptide of claim 30-35, wherein the modified IL-2 comprises a substitution at amino acid position T123.

37. — The polypeptide of claim 36, wherein the substitution is selected from T123D, T123E, T123H, T123K, T123N, T123Q, and T123R.

38. — The polypeptide of any one of claims 30-37, wherein the modified IL-2 comprises a substitution at amino acid position S127.

39. — The polypeptide of claim 38, wherein the substitution is selected from S127D, S127E, S127H, S127K, S127N, S127P, S127Q, and S127R.

40. — The polypeptide of any one of claims 30-39, wherein the modified IL-2 comprises a substitution at amino acid position S130.

41. — The polypeptide of claim 40, wherein the substitution is selected from S130D, S130E, S130H, S130K, S130N, S130P, S130Q, and S130R.

42. — The polypeptide of any one of claims 30-41, wherein the modified IL-2 comprises at least one substitution that reduces affinity for CD122 compared to wild-type IL-2.

43. — The polypeptide of claim 42, wherein the modified IL-2 comprises at least one substitution at at least one amino acid position selected from H16, L19, M23, D84, N88, and E95.

~~44. The polypeptide of claim 43, wherein the modified IL-2 comprises a substitution at amino acid position H16.~~

~~45. The polypeptide of claim 44, wherein the substitution is selected from H16A, H16T, H16V, and H16N.~~

~~46. The polypeptide of any one of claims 43-45, wherein the modified IL-2 comprises a substitution at amino acid position L19.~~

~~47. The polypeptide of claim 46, wherein the substitution is selected from L19A, L19P, L19Q, L19Y, L19N, L19S, L19T, L19V.~~

~~48. The polypeptide of any one of claims 43-47, wherein the modified IL-2 comprises a substitution at amino acid position M23.~~

~~49. The polypeptide of claim 48, wherein the substitution is selected from M23A, M23G, M23S, M23T, M23V, M23D, M23E, M23I, M23K, M23L, M23N, M23Q, M23R, and M23Y.~~

~~50. The polypeptide of any one of claims 43-49, wherein the modified IL-2 comprises a substitution at amino acid position D84.~~

~~51. The polypeptide of claim 50, wherein the substitution is selected from D84S, D84G, D84A, D84T, D84V, D84Y, and D84N.~~

~~52. The polypeptide of any one of claims 43-51, wherein the modified IL-2 comprises a substitution at amino acid position N88.~~

~~53. The polypeptide of claim 52, wherein the substitution is selected from N88T, N88A, and N88S.~~

~~54. The polypeptide of any one of claims 43-53, wherein the modified IL-2 comprises a substitution at amino acid position E95.~~

~~55. The polypeptide of claim 54, wherein the substitution is selected from E95Q, E95G, E95T, E95V, E95P, E95H, E95N, and E95Y.~~

~~56.9.~~ The polypeptide of any one of claims 1-~~558~~, wherein the modified IL-2 comprises at least one substitution that reduces affinity for CD25 compared to wild-type IL-2.

~~57.10.~~ The polypeptide of any one of claims 1-~~569~~, wherein the modified IL-2 comprises at least one substitution at at least one amino acid position selected from K43, Y45, E61, I114, P65, F42, R38, and L72, and/or wherein the modified IL-2 comprises a deletion of amino acid F42.

11. The polypeptide of claim 10, wherein the modified IL-2 comprises a substitution at amino acid position E61, optionally wherein the substitution is selected from E61R, E61G, E61H, E61N, E61P, E61S, E61T, E61Y, E61A, E61Q, and E61K.

12. The polypeptide of claim 10 or 11, wherein the modified IL-2 comprises a substitution at amino acid position P65, optionally wherein the substitution is selected from P65R, P65E, P65K, P65H, P65Y, P65Q, P65D, and P65N.

~~58-13. The polypeptide of any one of-claims 5710-12, wherein the modified IL-2 comprises: a substitution at amino acid F42 or comprises a deletion of amino acid F42.~~

~~a) a substitution at amino acid F42 optionally wherein the substitution is selected from F42K, F42A, F42R, F42G, F42S, and F42T or comprises a deletion of amino acid F42;~~

~~b) a substitution at amino acid position K43, optionally wherein the substitution is selected from K43E and K43D;~~

~~c) a substitution at amino acid position Y45, optionally wherein the substitution is selected from Y45R and Y45K;~~

~~d) a substitution at amino acid position I114, optionally wherein the substitution is selected from I114F, I114Y, and I114W;~~

~~e) a substitution at amino acid position R38, optionally wherein the substitution is selected from R38A and R38G; and /or~~

~~f) a substitution at amino acid position L72, optionally wherein the substitution is selected from L72 and L72G.~~

~~59. The polypeptide of claim 58, wherein the modified IL-2 comprise a substitution at amino acid position F42 selected from F42K, F42A, F42R, F42G, F42S, and F42T.~~

~~60. The polypeptide of claim 58, wherein the modified IL-2 comprise a deletion of amino acid F42.~~

~~61. The polypeptide of any one of claims 57-60, wherein the modified IL-2 comprises a substitution at amino acid position K43.~~

~~62. The polypeptide of claim 61, wherein the substitution is selected from K43E and K43D.~~

~~63. The polypeptide of any one of claims 57-62, wherein the modified IL-2 comprises a substitution at amino acid position Y45.~~

~~64. The polypeptide of claim 63, wherein the substitution is selected from Y45R and Y45K.~~

~~65. The polypeptide of any one of claims 57-64, wherein the modified IL-2 comprises a substitution at amino acid position E61.~~

~~66. The polypeptide of claim 65, wherein the substitution is selected from E61R, E61G, E61H, E61N, E61P, E61S, E61T, E61Y, E61A, E61Q, and E61K.~~

~~67. The polypeptide of any one of claims 57-66, wherein the modified IL-2 comprises a substitution at amino acid position H14.~~

~~68. The polypeptide of claim 67, wherein the substitution is H14F, H14Y, or H14W.~~

~~69. The polypeptide of any one of claims 57-68, wherein the modified IL-2 comprises a substitution at amino acid position P65.~~

~~70. The polypeptide of claim 69, wherein the substitution is selected from P65R, P65E, P65K, P65H, P65Y, P65Q, P65D, and P65N.~~

~~71. The polypeptide of any one of claims 57-70, wherein the modified IL-2 comprises a substitution at amino acid position R38.~~

~~72. The polypeptide of claim 71, wherein the substitution at R38 is selected from R38A and R38G.~~

~~73. The polypeptide of any one of claims 57-72, wherein the modified IL-2 comprises a substitution at amino acid position L72.~~

~~74. The polypeptide of claim 73, wherein the substitution at L72 is and L72G.~~

~~75.14.~~ The polypeptide of any one of claims 1-~~74~~13, wherein the modified IL-2 comprises substitution Q22A, or substitution R120A, or substitutions Q22A and R120A.

~~76.15.~~ The polypeptide of any one of claims 1-~~75~~14, wherein the modified IL-2 comprises substitutions P65R and R38A or substitution P65R and E61R.

~~77.16.~~ The polypeptide of any one of claims 1-~~76~~15, wherein the modified IL-2 comprises at least one substitution selected from H16A, L19A, L19N, M23A, ~~D84S~~ or D84Y, N88S, and E95Q.

~~78.17.~~ The polypeptide of any one of claims 1-~~77~~16, wherein the modified IL-2 comprises substitutions at amino acid positions P65, and H16, ~~and D84~~ optionally wherein the substitutions are P65R and H16A.

~~79. The polypeptide of claim 78, wherein the modified IL-2 comprises substitutions P65R, H16A, and D84S; or substitutions P65R, H16A, and D84Y.~~

~~80.18.~~ The polypeptide of any one of claims 1-~~79~~17, wherein the modified IL-2 comprises at least one substitution at at least one amino acid position selected from T3 and C125, optionally wherein the substitutions are selected from T3A, C125A, C125V, and C125S, and/or comprises a deletion of the first five amino acids of IL-2.

~~81. The polypeptide of claim 80, wherein the modified IL-2 comprises at least one substitution selected from T3A, C125A, C125V, and C125S.~~

~~82. The polypeptide of claim 81, wherein the modified IL-2 comprises T3A and C125S substitutions, or T3A and C125V substitutions.~~

~~83. The polypeptide of claim 81, wherein the modified IL-2 comprises a deletion of the first five amino acids of IL-2 and a C125S substitution or C125V substitution.~~

~~84.19.~~ The polypeptide of any one of the preceding claims, wherein the modified IL-2 comprises a set of substitutions selected from [T3A, H16A, E61R, P65R, D84Y, C125S], [T3A, H16A, M23T, E61R, P65R, D84Y, E95Q, C125S], [T3A, H16A, L19N, E61R, P65R, D84Y, C125S], [T3A, H16A, L19N, M23T, E61R, P65R, D84Y, E95Q, C125S], [T3A, H16A, E61R, P65R, D84Y, C125S, S127D], [T3A, H16A, M23T, E61R, P65R, D84Y, E95Q, C125S, S127D], [T3A, H16A, L19N, E61R, P65R, D84Y, C125S, S127D], and [T3A, H16A, L19N, M23T, E61R, P65R, D84Y, E95Q, C125S, S127D].

~~85.20.~~ A modified polypeptide comprising a modified IL-2, wherein the modified IL-2 comprises a set of substitutions selected from [T3A, H16A, E61R, P65R, D84Y, C125S], [T3A, H16A, M23T, E61R, P65R, D84Y, E95Q, C125S], [T3A, H16A, L19N, E61R, P65R, D84Y, C125S], [T3A, H16A, L19N, M23T, E61R, P65R, D84Y, E95Q, C125S], [T3A, H16A, E61R, P65R, D84Y, C125S, S127D], [T3A, H16A, M23T, E61R, P65R, D84Y, E95Q, C125S, S127D], [T3A, H16A, L19N, E61R, P65R, D84Y, C125S, S127D], and [T3A, H16A, L19N, M23T, E61R, P65R, D84Y, E95Q, C125S, S127D].

~~86.21.~~ The polypeptide of any one of the preceding claims, wherein the modified IL-2 comprises the indicated substitutions, and does not comprise any additional substitutions.

~~87.22.~~ The polypeptide of any one of the preceding claims, wherein the modified IL-2 is a modified human IL-2.

~~88. —The polypeptide of any one of the preceding claims, wherein the amino acid positions correspond to the amino acid positions in SEQ ID NO: 1.~~

~~89.23.~~ The polypeptide of any one of the preceding claims, wherein the modified IL-2 comprises an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 84 and ~~comprising~~ comprises corresponding substitutions of an amino acid sequence selected from SEQ ID NOs: 105-290105-107, 249, 251, 253, 255, 257, 259, 261, and 283-290 and comprises substitution D84Y.

~~90.24.~~ The polypeptide of any one of the preceding claims, wherein the modified IL-2 comprises an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence selected from SEQ ID NOs: 160 and 270-277 and comprises substitution D84Y.

~~91. —The polypeptide of any one of the preceding claims, wherein the modified IL-2 comprises an amino acid sequence selected from SEQ ID NOs: 105-290.~~

~~92.25.~~ The polypeptide of any one of the preceding claims, wherein the modified IL-2 comprises an amino acid sequence selected from SEQ ID NOs: 160 and 270-277.

~~93.26.~~ The polypeptide of any one of the preceding claims, wherein the polypeptide comprises an Fc region, optionally wherein the modified IL-2 is fused to the N-terminus or the C-terminus of the Fc region.

~~94. —The polypeptide of claim 93, wherein the modified IL-2 is fused to the N-terminus or the C-terminus of the Fc region.~~

~~95.27.~~ The polypeptide of claim ~~93 or claim 94~~26, wherein the Fc region comprises a substitution at Kabat amino acid position T366, or wherein the Fc region comprises at least one substitution at at least one Kabat amino acid position selected from T366, L368, and Y407; optionally wherein the Fc region comprises a T366W substitution or wherein the Fc region comprises T366S, L368A, and Y407V mutations.

~~96. —The polypeptide of claim 95, wherein the Fc region comprises a T366W substitution.~~

~~97. —The polypeptide of claim 93 or claim 94, wherein the Fc region comprises at least one substitution at at least one Kabat amino acid position selected from T366, L368, and Y407.~~

~~98. —The polypeptide of claim 108, wherein the Fc region comprises T366S, L368A, and Y407V mutations.~~

~~99.28.~~ The polypeptide of ~~any one of claims 93-98~~claim 26 or 27, wherein the Fc region comprises a substitution at a Kabat position selected from S354 and Y349,optionally wherein the Fc region comprises a S354C or a Y349C substitution.

~~100.—~~The polypeptide of claim 99, wherein the Fc region comprises a S354C or a Y349C substitution.

~~101.29.~~ The polypeptide of any one of claims ~~93-100~~26-28, wherein the Fc region comprises a substitution at Kabat amino acid position H435,optionally wherein the Fc region comprises a substitution selected from H435R and H435K.

~~102.—~~The polypeptide of claim 101, wherein the Fc region comprises a substitution selected from H435R and H435K.

~~103.30.~~ The polypeptide of any one of claims ~~93-102~~26-29, wherein the Fc region comprises at least one substitution at at least one Kabat amino acid position selected from M252 and M428,optionally wherein the Fc region comprises M252Y and M428V substitutions.

~~104.—~~The polypeptide of claim 103, wherein the Fc region comprises M252Y and M428V substitutions.

~~105.31.~~ The polypeptide of any one of claims ~~93-104~~26-30, wherein the Fc region comprises a deletion of Kabat amino acids E233, L234, and L235;or wherein the Fc region comprises at least one substitution at at least one amino acid position selected from L234, L235, and P329, optionally wherein the Fc region comprises L234A, L235A, and P329G substitutions.

~~106.—~~The polypeptide of any one of claims 93-104, wherein the Fc region comprises at least one substitution at at least one amino acid position selected from L234, L235, and P329.

~~107.—~~The polypeptide of claim 106, wherein the Fc region comprises L234A, L235A, and P329G substitutions.

~~108.32.~~ The polypeptide of any one of claims ~~93-107~~26-31, wherein the Fc region comprises an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence selected from SEQ ID Nos: 47-83, 292, and 293.

~~109.33.~~ The polypeptide of any one of claims ~~93-108~~26-32, wherein the Fc region is part of a heavy chain constant region,optionally wherein the heavy chain constant region is an IgG1, IgG2, IgG3, or IgG4 constant region.

~~110. The polypeptide of claim 109, wherein the heavy chain constant region is an IgG constant region.~~

~~111. The polypeptide of claim 110, wherein the heavy chain constant region is an IgG1, IgG2, IgG3, or IgG4 constant region.~~

~~112. The polypeptide of any one of claims 93-111, wherein the modified IL-2 is fused to the C-terminus of the Fc region or heavy chain constant region.~~

~~113.~~34. The polypeptide of claim ~~112~~26-33, wherein the modified IL-2 is fused to the C-terminus of the Fc region or heavy chain constant region via a linker comprising 1-20 amino acids, optionally wherein the linker comprises glycine amino acids or glycine and serine amino acids.

~~114. The polypeptide of claim 113, wherein the linker comprises glycine amino acids.~~

~~115. The polypeptide of claim 114, wherein the linker comprises glycine and serine amino acids.~~

~~116. The polypeptide of any one of claims 113-115, wherein a majority, or all, of the amino acids in the linker are glycine and serine.~~

~~117.~~35. The polypeptide of any one of claims ~~93-116~~26-34, wherein the polypeptide comprises an amino acid sequence selected from SEQ ID NOs: ~~105-290~~105-107, 249, 251, 253, 255, 257, 259, 261, and 283-290 and comprises substitution D84Y, and an amino acid sequence selected from SEQ ID NOs: 48, 64, 292, and 293.

~~118.~~36. The polypeptide of any one of the preceding claims, wherein the polypeptide comprises at least one antigen binding domain, or wherein the polypeptide comprises two, three, or four antigen binding domains.

~~119. The polypeptide of claim 118, wherein the polypeptide comprises two, three, or four antigen binding domains.~~

~~120.~~37. The polypeptide of claim ~~118 or claim 119~~36, wherein at least one antigen binding domain specifically binds to a T-cell antigen or a natural killer cell antigen.

~~121.~~38. The polypeptide of ~~any one of claims 118-120~~claim 36 or 37, wherein at least one antigen binding domain specifically binds to a CD4⁺ T-cell antigen or a CD8⁺ T-cell antigen, optionally wherein the at least one antigen binding domain specifically binds to an antigen on an activated CD4⁺ T-cell or an activated CD8⁺ T-cell.

~~122.—The polypeptide of claim 121, wherein the at least one antigen binding domain specifically binds to an antigen on an activated CD4⁺ T-cell or an activated CD8⁺ T-cell.~~

~~123.—The polypeptide of any one of claims 118–122, wherein at least one antigen binding domain is an agonist.~~

~~124.—The polypeptide of any one of claims 118–122, wherein the antigen binding domain is an antagonist.~~

~~125.39.~~ The polypeptide of any one of claims ~~118–124~~36–38, wherein at least one antigen binding domain specifically binds to PD-1, CTLA-4, LAG3, TIM3, 4-1BB, OX40, GITR, CD8a, CD8b, CD4, Nkp30, NKG2A, TIGIT, TGFβR1, TGFβR2, Fas, NKG2D, Nkp46, PD-L1, CD107a, ICOS, TNFR2, CD16a, or γδTCR.

~~126.—The polypeptide of any one of claims 118–124, wherein at least one antigen binding domain specifically binds to PD-1.~~

~~127.—The polypeptide of any one of claims 118–126, wherein at least one antigen binding domain is a human or humanized antigen binding domain.~~

~~128.—The polypeptide of claim 127, wherein each antigen binding domain is, independently, a human or humanized antigen binding domain.~~

~~129.40.~~ The polypeptide of any one of claims ~~118–128~~36–39, wherein at least one antigen binding domain, or each antigen binding domain, comprises a VHH domain, a VH domain and a VL domain, or a single chain Fv (scFv).

~~130.—The polypeptide of claim 129, wherein each antigen binding domain comprises a VHH domain.~~

~~131.—The polypeptide of any one of claims 118–128, wherein at least one antigen binding domain comprises a VH domain and a VL domain.~~

~~132.41.~~ The polypeptide of claim ~~131~~36–40, wherein at least one antigen binding domain comprises the VH domain and the VL domain of an antibody selected from pembrolizumab, nivolumab, AMP-514, TSR-042, STI-A1110, ipilimumab, tremelimumab, urelumab, utomilumab, atezolizumab, and durvalumab.

~~133.—The polypeptide of claim 131 or 132, wherein the at least one antigen binding domain comprises a single chain Fv (scFv).~~

~~134.42.~~ The polypeptide of claim ~~131 or 132~~36–41, wherein the polypeptide comprises at least one antigen binding domain comprising a VH domain and a VL domain.

wherein the polypeptide comprises a heavy chain constant region, wherein the VH domain is fused to the heavy chain constant region, and wherein the VL domain is associated with the VH domain, optionally wherein the VL domain is fused to a light chain constant region, optionally wherein the light chain constant region is selected from kappa and lambda.

~~135.—The polypeptide of claim 134, wherein the VL domain is fused to a light chain constant region.~~

~~136.—The polypeptide of claim 135, wherein the light chain constant region is selected from kappa and lambda.~~

~~137.43.~~ The polypeptide of any one of claims ~~118-136~~36-42, wherein each of the antigen binding domains are the same and/or wherein each of the antigen binding domains specifically binds to the same antigen, or wherein at least one of the antigen binding domains specifically binds to a different antigen than at least one of the other antigen binding domains.

~~138.—The polypeptide of any one of claims 118-137, wherein each of the antigen binding domains specifically bind to the same antigen.~~

~~139.—The polypeptide of any one of claims 118-136, wherein at least one of the antigen binding domains specifically binds to a different antigen than at least one of the other antigen binding domains.~~

~~140.44.~~ The polypeptide of claim ~~139~~36-43, wherein at least one antigen binding domain specifically binds to PD-1 and at least one other antigen binding domain specifically binds to a T-cell antigen or natural killer cell antigen other than PD-1.

~~141.—The polypeptide of any one of claims 118-140, wherein at least one antigen binding domain binds to PD-1, CTLA-4, LAG3, TIM3, 4-1BB, OX40, GITR, CD8a, CD8b, CD4, NKp30, NKG2A, TIGIT, TGFβR1, TGFβR2, Fas, NKG2D, NKp46, PD-L1, CD107a, ICOS, TNFR2, CD16a, DNAM1, or γδTCR (Vγ9, Vγ2, Vδ1).~~

~~142.45.~~ The polypeptide of any one of claims ~~93-141~~36-44, wherein the polypeptide forms a homodimer or a heterodimer under physiological conditions.

~~143.46.~~ The polypeptide of any one of the preceding claims, wherein the modified IL-2 binds a human IL-2R with an affinity at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, at least 10-fold, at least 20-fold, at least 30-fold, at least 50-fold, or at least 100-fold lower than the affinity of human wild type IL-2 for the IL-2R.

~~144.47.~~ A complex comprising a first polypeptide and a second polypeptide, wherein the first polypeptide is the polypeptide of any one of the preceding claims.

~~145.48.~~ The complex of claim ~~144~~47, wherein the first polypeptide comprises a first Fc region and the second polypeptide comprises a second Fc region, optionally wherein each Fc region is an isotype selected from human IgG1, IgG2, IgG3, and IgG4.

~~146.~~ The complex of claim ~~144~~ or claim ~~145~~, wherein each Fc region is an isotype selected from human IgG1, IgG2, IgG3, and IgG4.

~~147.~~ The complex of claim ~~146~~, wherein each Fc region is a human IgG1.

~~148.49.~~ The complex of ~~any one of claims 144-147~~claim 47 or 48, wherein each Fc region comprises a deletion of amino acids E233, L234, and L235; and/or wherein each Fc region comprises a H435R or H435K mutation; and/or wherein each Fc region comprises a mutations M252Y and M428L or mutations M252Y and M428V; and/or wherein the first Fc region or the second Fc region comprises a T366W mutation, and the other Fc region comprises mutations T366S, L368A, and Y407V; and/or wherein the first Fc region or the second Fc region comprises a S354C mutation.

~~149.~~ The complex of ~~any one of claims 144-148~~, wherein each Fc region comprises a ~~H435R or H435K mutation.~~

~~150.~~ The complex of ~~any one of claims 155-160~~, wherein the Fc region comprises a ~~mutations M252Y and M428L or mutations M252Y and M428V.~~

~~151.~~ The complex of ~~any one of claims 144-150~~, wherein the first Fc region or the second Fc region comprises a ~~T366W mutation, and the other Fc region comprises mutations T366S, L368A, and Y407V.~~

~~152.~~ The complex of claim ~~151~~, wherein the first Fc region or the second Fc region comprises a ~~S354C mutation.~~

~~153.50.~~ The complex of any one of claims ~~144-152~~47-49, wherein each Fc region independently comprises an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence selected from SEQ ID Nos: 47-83, 292, and 293.

~~154.51.~~ The complex of any one of claims ~~144-153~~47-50, wherein the second polypeptide does not comprise a modified IL-2.

~~155.—The complex of any one of claims 144-154, wherein the first polypeptide comprises at least one antigen binding domain.~~

~~156.—The complex of any one of claims 144-155, wherein the second polypeptide comprises at least one antigen binding domain.~~

~~157.—The complex of any one of claims 144-156, wherein the first polypeptide comprises a first antigen binding domain, an Fc region, and a modified IL-2.~~

~~158.52.~~ The complex of claim ~~157~~47-51, wherein the first polypeptide comprises a first antigen binding domain, an Fc region, and a modified IL-2 and the first antigen binding domain is fused to the N-terminus of the Fc region and the modified IL-2 is fused to the C-terminus of the Fc region.

~~159.—The complex of claim 157 or claim 158, wherein the second polypeptide comprises a second antigen binding domain and an Fc region.~~

~~160.—The complex of claim 159, wherein the first antigen binding domain and the second antigen binding domain are the same or different.~~

~~161.53.~~ The complex of claim ~~160~~52, wherein the second polypeptide comprises a second antigen binding domain and wherein:

- a) the first antigen binding domain and the second antigen binding domain both bind PD-1;
- b) the first antigen binding domain binds PD-1, and the second antigen binding domain binds LAG3;
- c) the first antigen binding domain binds PD-1, and the second antigen binding domain binds CTLA-4;
- d) the first antigen binding domain binds PD-1, and the second antigen binding domain binds 4-1BB;
- e) the first antigen binding domain binds PD-1, and the second antigen binding domain binds OX40;
- f) the first antigen binding domain binds PD-1, and the second antigen binding domain binds GITR;
- g) the first antigen binding domain binds PD-1, and the second antigen binding domain binds CD8a;

- h) the first antigen binding domain binds PD-1, and the second antigen binding domain binds CD8b;
- i) the first antigen binding domain binds PD-1, and the second antigen binding domain binds CD4;
- j) the first antigen binding domain binds PD-1, and the second antigen binding domain binds NKp30;
- k) the first antigen binding domain binds PD-1, and the second antigen binding domain binds NKG2A;
- l) the first antigen binding domain binds PD-1, and the second antigen binding domain binds TIGIT;
- m) the first antigen binding domain binds PD-1, and the second antigen binding domain binds NKG2D;
- n) the first antigen binding domain binds PD-1, and the second antigen binding domain binds TGFBR2;
- o) the first antigen binding domain binds PD-1, and the second antigen binding domain binds Fas;
- p) the first antigen binding domain binds PD-1, and the second antigen binding domain binds CD107a;
- q) the first antigen binding domain binds PD-1, and the second antigen binding domain binds NKp46;
- r) the first antigen binding domain binds CD8a, and the second antigen binding domain binds TGFR β R2;
- s) the first antigen binding domain binds CD8a, and the second antigen binding domain binds Fas;
- t) the first antigen binding domain binds NKG2D, and the second antigen binding domain binds TGFR β R2;
- u) the first antigen binding domain binds NKG2D, and the second antigen binding domain binds Fas;
- v) the first antigen binding domain binds NKG2A, and the second antigen binding domain binds TGFR β R2;

- w) the first antigen binding domain binds NKG2A, and the second antigen binding domain binds Fas;
- x) the first antigen binding domain binds NKp46, and the second antigen binding domain binds TGFR β R2;
- y) the first antigen binding domain binds NKp46, and the second antigen binding domain binds Fas;
- z) the first antigen binding domain binds CTLA-4, and the second antigen binding domain binds LAG3;
- aa) the first antigen binding domain binds CTLA-4, and the second antigen binding domain binds Tim3;
- bb) the first antigen binding domain binds CTLA-4, and the second antigen binding domain binds OX40;
- cc) the first antigen binding domain binds CTLA-4, and the second antigen binding domain binds GITR;
- dd) the first antigen binding domain binds CTLA-4, and the second antigen binding domain binds CD107a;
- ee) the first antigen binding domain binds CTLA-4, and the second antigen binding domain binds NKp46
- ff) the first antigen binding domain binds ICOS, and the second antigen binding domain binds TNFR2;
- gg) the first antigen binding domain binds $\gamma\delta$ TCR, and the second antigen binding domain binds NKG2D;
- hh) the first antigen binding domain binds $\gamma\delta$ TCR, and the second antigen binding domain binds DNAM1;
- ii) the first antigen binding domain binds $\gamma\delta$ TCR, and the second antigen binding domain binds TIGIT;
- jj) the first antigen binding domain binds $\gamma\delta$ TCR, and the second antigen binding domain binds 4-1BB;
- kk) the first antigen binding domain binds $\gamma\delta$ TCR, and the second antigen binding domain binds Fas;

ll) the first antigen binding domain binds $\gamma\delta$ TCR, and the second antigen binding domain binds NKG2A; or

mm) the first antigen binding domain binds $\gamma\delta$ TCR, and the second antigen binding domain binds CD16a.

~~162. The complex of any one of claims 144-161, wherein the modified IL-2 binds a human IL-2R with an affinity at least 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, at least 10 fold, at least 20 fold, at least 30 fold, at least 50 fold, or at least 100 fold lower than the affinity of human wild type IL-2 for the IL-2R.~~

~~163.54.~~ A pharmaceutical composition comprising a polypeptide of any one of claims 1-~~154~~46 or the complex of any one of claims ~~144-162~~47-53 and a pharmaceutically acceptable carrier.

~~164.55.~~ An isolated nucleic acid the encodes a polypeptide of any one of claims 1-~~143~~46 or the complex of any one of claims ~~144-162~~47-53.

~~165.56.~~ An expression vector comprising the nucleic acid of claim ~~164~~55.

~~166.57.~~ An isolated host cell comprising the nucleic acid of claim ~~164~~55 or the expression vector of claim ~~165~~56.

~~167.58.~~ An isolated host cell that expresses the polypeptide of any one of claims 1-~~143~~46 or the complex of any one of claims ~~144-162~~47-53.

~~168.59.~~ A method of producing the polypeptide of any one of claims 1-~~143~~46 or the complex of any one of claims ~~144-162~~47-53 comprising incubating the host cell of claim ~~166~~57 or claim ~~167~~58 under conditions suitable to express the polypeptide or complex, and optionally isolating the polypeptide of complex.

~~169. The method of claim 168, further comprising isolating the polypeptide or complex.~~

~~170.60.~~ A method of increasing CD4+ and/or CD8+ T cell proliferation comprising contacting T cells with the polypeptide of any one of claims 1-~~154~~46 or the complex of any one of claims ~~144-162~~47-53, wherein the CD4+ and/or CD8+ T cells are in vitro or in vivo.

~~171. The method of claim 170, wherein the CD4+ and/or CD8+ T cells are in vitro.~~

~~172. The method of claim 170, wherein the CD4+ and/or CD8+ T cells are in vivo.~~

~~173. —The method of any one of claims 170-172, wherein the increase is at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.~~

~~174.61.~~ _____ A method of increasing NK cell proliferation comprising contacting NK cells with the polypeptide of any one of claims 1-~~143~~ 46 or the complex of any one of claims ~~144-162~~ 47-53.

~~175. —The method of claim 174, wherein the increase is at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.~~

~~176.62.~~ _____ ~~A method of treating cancer comprising administering to a subject with cancer a pharmaceutically effective amount~~ Use of the polypeptide of any one of claims 1-~~143~~ 46 or the complex of any one of claims ~~144-162~~ 47-53, or the pharmaceutical composition of claim ~~163~~ 54, in the manufacture of a medicament for treating cancer in a subject with cancer.

~~177.63.~~ _____ The ~~use~~ method of claim ~~176~~ 62, wherein the cancer is selected from basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer; gastrointestinal cancer; glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; liver cancer; lung cancer; small-cell lung cancer; non-small cell lung cancer; adenocarcinoma of the lung; squamous carcinoma of the lung; melanoma; myeloma; neuroblastoma; oral cavity cancer; ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma; Hodgkin's lymphoma; non-Hodgkin's lymphoma; B-cell lymphoma; low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; Waldenstrom's macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; and chronic myeloblastic leukemia.

~~178.64.~~ 178.64. The ~~use~~method of claim ~~176.62~~ or ~~177.63~~, ~~further comprising administering wherein~~ an additional therapeutic agent ~~is to be adminisitered, optionally wherein~~ the additional therapeutic agent is an anti-cancer agent.

~~179.~~ The method of claim 178, wherein the additional therapeutic agent is an anti-cancer agent.

~~180.65.~~ 180.65. The method of claim ~~179.64~~, wherein the anti-cancer agent is selected from a chemotherapeutic agent, an anti-cancer biologic, radiation therapy, CAR-T therapy, and an oncolytic virus, optionally wherein the additional therapeutic agent is an anti-cancer biologic.

~~181.~~ The method of claim 179 or claim 180, wherein the additional therapeutic agent is an anti-cancer biologic.

~~182.~~ The method of claim 181, wherein the anti-cancer biologic is an agent that inhibits PD-1 and/or PD-L1.

~~183.~~ The method of claim 181, wherein the anti-cancer biologic is an agent that inhibits VISTA, gpNMB, B7H3, B7H4, HHLA2, CTLA4, or TIGIT.

~~184.~~ The method of any one of claims 179-183, wherein the anti-cancer agent is an antibody.

~~185.~~ The method of claim 181, wherein the anti-cancer biologic is a cytokine.

~~186.~~ The method of claim 179, wherein the anti-cancer agent is CAR-T therapy.

~~187.~~ The method of claim 179, wherein the anti-cancer agent is an oncolytic virus.

~~188.66.~~ 188.66. The ~~method~~use of any one of claims ~~176.62-187.65~~, further comprising tumor resection and/or radiation therapy.