

CLAIMS

1. An antigen-binding site that binds PD-L1, comprising:
 - a heavy chain variable domain (VH) comprising a complementarity-determining region 1 (CDR1) sequence of SEQ ID NO: 11, 3, 19, 33, 52, or 63; complementarity-determining region 2 (CDR2) sequence of SEQ ID NO: 12, 4, 20, 34, 41, 53, or 64; and complementarity-determining region 3 (CDR3) sequence of SEQ ID NO: 89, 5, 13, 21, 27, 35, 46, 54, 65, 71, 74, 77, 80, 83, or 86; and
 - a light chain variable domain (VL) comprising CDR1 sequence of SEQ ID NO: 47, 6, 14, 22, 28, 36, 55, or 66; CDR2 sequence of SEQ ID NO: 48, 7, 15, 23, 29, 37, 42, 56, 60, or 67; and CDR3 sequences of SEQ ID NO: 49, 8, 16, 24, 30, 38, 43, 57, or 68.
2. The antigen-binding site of claim 1, comprising:
 - (a) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 11, 12, and 89, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 47, 48, and 49, respectively
 - (b) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 3, 4, and 5, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 6, 7, and 8, respectively;
 - (c) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 11, 12, and 13, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 14, 15, and 16, respectively;
 - (d) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 19, 20, and 21, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 22, 23, and 24, respectively;
 - (e) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 19, 20, and 27, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 28, 29, and 30, respectively;
 - (f) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 33, 34, and 35, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 36, 37, and 38, respectively;

(g) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 3, 41, and 5, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 6, 42, and 43, respectively;

(h) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 11, 12, and 46, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 47, 48, and 49, respectively;

(i) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 52, 53, and 54, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 55, 56, and 57, respectively;

(j) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 11, 12, and 46, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 47, 60, and 49, respectively;

(k) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 63, 64, and 65, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 66, 67, and 68, respectively;

(l) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 11, 12, and 71, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 47, 48, and 49, respectively;

(m) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 11, 12, and 74, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 47, 48, and 49, respectively;

(n) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 11, 12, and 77, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 47, 48, and 49, respectively;

(o) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 11, 12, and 80, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 47, 48, and 49, respectively;

(p) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 11, 12, and 83, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 47, 48, and 49, respectively; or

(q) VH comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 11, 12, and 86, respectively; and a VL comprising CDR1, CDR2, and CDR3 sequences of SEQ ID NOS: 47, 48, and 49, respectively.

3. The antigen-binding site of claim 1 or 2, wherein:

(a) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:87 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:88;

(b) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:1 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:2;

(c) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:9 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:10;

(d) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:17 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:18;

(e) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:25 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:26;

(f) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:31 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:32;

(g) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:39 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:40;

(h) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:44 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:45;

(i) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:50 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:51;

(j) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:58 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:59;

(k) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:61 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:62;

(l) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:69 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:70;

(m) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:72 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:73;

(n) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:75 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:76;

(o) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:78 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:79;

(p) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:81 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:82; or

(q) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:84 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:85.

4. The antigen-binding site of any one of claims 1-3, wherein:

(a) the VH comprises or consists of the amino acid sequence of SEQ ID NO:87 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:88

(b) the VH comprises or consists of the amino acid sequence of SEQ ID NO:1 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:2;

(c) the VH comprises or consists of the amino acid sequence of SEQ ID NO:9 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:10;

(d) the VH comprises or consists of the amino acid sequence of SEQ ID NO:17 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:18;

(e) the VH comprises or consists of the amino acid sequence of SEQ ID NO:25 and the VL comprises the amino acid sequence of SEQ ID NO:26;

(f) the VH comprises or consists of the amino acid sequence of SEQ ID NO:31 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:32;

(g) the VH comprises or consists of the amino acid sequence of SEQ ID NO:39 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:40;

(h) the VH comprises or consists of the amino acid sequence of SEQ ID NO:44 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:45;

(i) the VH comprises or consists of the amino acid sequence of SEQ ID NO:50 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:51;

(j) the VH comprises or consists of the amino acid sequence of SEQ ID NO:58 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:59;

(k) the VH comprises or consists of the amino acid sequence of SEQ ID NO:61 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:62;

(l) the VH comprises or consists of the amino acid sequence of SEQ ID NO:69 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:70;

(m) the VH comprises or consists of the amino acid sequence of SEQ ID NO:72 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:73;

(n) the VH comprises or consists of the amino acid sequence of SEQ ID NO:75 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:76;

(o) the VH comprises or consists of the amino acid sequence of SEQ ID NO:78 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:79;

(p) the VH comprises or consists of the amino acid sequence of SEQ ID NO:81 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:82; or

(q) the VH comprises or consists of the amino acid sequence of SEQ ID NO:84 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:85.

5. The antigen-binding site of any one of claims 1-4, wherein the antigen-binding site is a single-chain fragment variable fragment (scFv).
6. The antigen-binding site of any one of claims 1-5, wherein the antigen-binding site is an antigen-binding fragment (Fab).
7. The antigen-binding site of any one of claims 1-6, wherein the antigen-binding site binds human PD-L1 with an K_D less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 0.8 nM, less than about 0.6 nM, less than about 0.4 nM, less than about 0.2 nM, or less than about 0.1 nM, as measured by surface plasmon resonance (SPR).
8. The antigen-binding site of any one of claims 1-7, wherein the antigen-binding site binds human PD-L1 with an EC_{50} less than about 160 nM, less than about 10 nM, less than about 1.5 nM, less than about 1.2 nM, less than about 1.0 nM, less than about 0.8 nM, less than about 0.7 nM, less than about 0.6 nM, less than about 0.5

nM, or less than about 0.4 nM, as measured by enzyme-linked immunosorbent assay (ELISA).

9. The antigen-binding site of any one of claims 1-8, wherein the antigen-binding site binds cells expressing human PD-L1 with an EC₅₀ less than about 40 nM, less than about 10 nM, less than about 8 nM, less than about 6 nM, less than about 4 nM, less than about 2 nM, less than about 1 nM, less than about 0.5 nM, less than about 0.3 nM, less than about 0.2 nM, less than about 0.1 nM, as measured by fluorescence-activated cell sorting (FACS).
10. The antigen-binding site of any one of claims 1-9, wherein the antigen-binding site competes with PD-1 for binding PD-L1 or inhibits PD-L1 binding to PD-1.
11. A protein comprising the antigen-binding site of any one of claims 1-10.
12. The protein of claim 11, comprising one or more antibody heavy chain constant regions.
13. The protein of claim 12, wherein the antibody heavy chain constant region is human IgG heavy chain constant region.
14. The protein of claim 12 or 13, wherein the antibody heavy chain constant region is human IgG1 heavy chain constant region.
15. The protein of any one of claims 12-14, wherein the antibody heavy chain constant region comprises an amino acid sequence at least 90% identical to SEQ ID NO:90.
16. The protein of any one of claims 12-15, wherein the antibody heavy chain constant region comprises, relative to SEQ ID NO:90, one or more mutations selected from L234A, L235A, P329G, Y349C, S354C, T366S, T366W, L368A, F405K, K409A and Y407V, numbered according to the EU numbering system.
17. The protein of any one of claims 12-16, wherein the antibody heavy chain constant region comprises an amino acid sequence of SEQ ID NO:91 or SEQ ID NO:92.

18. The protein of any one of claims 12-17, wherein the protein comprises a first antibody heavy chain constant region comprising, relative to SEQ ID NO:90, one or more mutations selected from S354C, T366W and K409A and a second antibody heavy chain constant region comprising, relative to SEQ ID NO:90, one or more mutations selected from Y349C, T366S, L368A, F405K and Y407V, numbered according to the EU numbering system.

19. The protein of any one of claims 11-18, wherein the protein is an antibody.

20. The protein of claim 19, wherein the antibody comprises:

(a) a heavy chain (HC) comprising an amino acid sequence at least 90% identical to SEQ ID NO:148 and a light chain (LC) comprising an amino acid sequence at least 90% identical to SEQ ID NO:149;

(b) a HC comprising an amino acid sequence at least 90% identical to SEQ ID NO:150 and a LC comprising an amino acid sequence at least 90% identical to SEQ ID NO:151;

(c) a HC comprising an amino acid sequence at least 90% identical to SEQ ID NO:152 and a LC comprising an amino acid sequence at least 90% identical to SEQ ID NO:153;

(d) a HC comprising an amino acid sequence at least 90% identical to SEQ ID NO:154 and a LC comprising an amino acid sequence at least 90% identical to SEQ ID NO:155;

(e) a HC comprising an amino acid sequence at least 90% identical to SEQ ID NO:156 and a LC comprising an amino acid sequence at least 90% identical to SEQ ID NO:157; or

(f) a HC comprising an amino acid sequence at least 90% identical to SEQ ID NO:159 and a LC comprising an amino acid sequence at least 90% identical to SEQ ID NO:160.

21. The protein of claim 19 or 20, wherein the antibody comprises:

(a) a HC comprising or consists of the amino acid sequence of SEQ ID NO:148 and a LC comprising or consists of the amino acid sequence of SEQ ID NO:149;

(b) a HC comprising or consists of the amino acid sequence of SEQ ID NO:150 and a LC comprising or consists of the amino acid sequence of SEQ ID NO:151;

(c) a HC comprising or consists of the amino acid sequence of SEQ ID NO:152 and a LC comprising or consists of the amino acid sequence of SEQ ID NO:153;

(d) a HC comprising or consists of the amino acid sequence of SEQ ID NO:154 and a LC comprising or consists of the amino acid sequence of SEQ ID NO:155;

(e) a HC comprising or consists of the amino acid sequence of SEQ ID NO:156 and a LC comprising or consists of the amino acid sequence of SEQ ID NO:157; or

(f) a HC comprising or consists of the amino acid sequence of SEQ ID NO:159 and a LC comprising or consists of the amino acid sequence of SEQ ID NO:160.

22. The protein of any one of claims 19-21, wherein the antibody binds human PD-L1 with an K_D of less than about 10 nM, less than about 6 nM, less than about 3 nM, less than about 1 nM, or less than about 0.4 nM, as measured by SPR.
23. The protein of any one of claims 19-22, wherein the antibody binds human PD-L1 with an EC_{50} of less than about 0.2 nM, as measured by ELISA.
24. The protein of any one of claims 19-23, wherein the antibody binds cells expressing human PD-L1 with an EC_{50} of less than about 7 nM, as measured by FACS.
25. The protein of any one of claims 19-24, wherein the antibody inhibits tumor growth *in vivo*.

26. The protein of any one of claims 19-25, wherein the antibody induces IFN γ and TNF α secretion *in vivo*, at a comparable to or more enhanced level as compared to atezolizumab.

27. A bifunctional protein, comprising:

(a) an antigen-binding site that binds PD-L1, comprising:

(i) a heavy chain variable domain (VH) comprising complementarity-determining region 1 (CDR1) of SEQ ID NO: 11, 3, 19, 33, 52, or 63; complementarity-determining region 2 (CDR2) of SEQ ID NO: 12, 4, 20, 34, 41, 53, or 64; and complementarity-determining region 3 (CDR3) of SEQ ID NO: 89, 5, 13, 21, 27, 35, 46, 54, 65, 71, 74, 77, 80, 83, or 86; and

(ii) a light chain variable domain (VL) comprising CDR1 sequence of SEQ ID NO: 47, 6, 14, 22, 28, 36, 55, or 66; CDR2 sequence of SEQ ID NO: 48, 7, 15, 23, 29, 37, 42, 56, 60, or 67; and CDR3 sequences of SEQ ID NO: 49, 8, 16, 24, 30, 38, 43, 57, or 68; and

(b) an interleukin-15 (IL-15) polypeptide, an interleukin-15 receptor alpha (IL-15R α) polypeptide, a wild-type interleukin-2 (IL-2) polypeptide, or an engineered IL-2 polypeptide, or a functional fragment or variant thereof.

28. The bifunctional protein of claim 27, further comprising one or more antibody heavy chain constant regions.

29. The bifunctional protein of any one of claims 27-28, wherein the antigen-binding site that binds PD-L1 comprises:

(a) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:87 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:88;

(b) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:1 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:2;

(c) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:9 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:10;

(d) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:17 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:18;

(e) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:25 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:26;

(f) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:31 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:32;

(g) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:39 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:40;

(h) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:44 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:45;

(i) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:50 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:51;

(j) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:58 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:59;

(k) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:61 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:62;

(l) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:69 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:70;

(m) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:72 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:73;

(n) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:75 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:76;

(o) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:78 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:79;

(p) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:81 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:82; or

(q) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:84 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:85.

30. The bifunctional protein of any one of claims 27-29, wherein the antigen-binding site that binds PD-L1 comprises:

(a) the VH comprises or consists of the amino acid sequence of SEQ ID NO:87 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:88

(b) the VH comprises or consists of the amino acid sequence of SEQ ID NO:1 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:2;

(c) the VH comprises or consists of the amino acid sequence of SEQ ID NO:9 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:10;

(d) the VH comprises or consists of the amino acid sequence of SEQ ID NO:17 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:18;

(e) the VH comprises or consists of the amino acid sequence of SEQ ID NO:25 and the VL comprises the amino acid sequence of SEQ ID NO:26;

(f) the VH comprises or consists of the amino acid sequence of SEQ ID NO:31 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:32;

(g) the VH comprises or consists of the amino acid sequence of SEQ ID NO:39 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:40;

(h) the VH comprises or consists of the amino acid sequence of SEQ ID NO:44 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:45;

(i) the VH comprises or consists of the amino acid sequence of SEQ ID NO:50 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:51;

(j) the VH comprises or consists of the amino acid sequence of SEQ ID NO:58 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:59;

(k) the VH comprises or consists of the amino acid sequence of SEQ ID NO:61 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:62;

(l) the VH comprises or consists of the amino acid sequence of SEQ ID NO:69 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:70;

(m) the VH comprises or consists of the amino acid sequence of SEQ ID NO:72 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:73;

(n) the VH comprises or consists of the amino acid sequence of SEQ ID NO:75 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:76;

(o) the VH comprises or consists of the amino acid sequence of SEQ ID NO:78 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:79;

(p) the VH comprises or consists of the amino acid sequence of SEQ ID NO:81 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:82; or

(q) the VH comprises or consists of the amino acid sequence of SEQ ID NO:84 and the VL comprises or consists of the amino acid sequence of SEQ ID NO:85.

31. The bifunctional protein of any one of claims 27-30, comprising:

(a) a first subunit comprising the antigen-binding site that binds PD-L1, an interleukin-15 (IL-15) polypeptide or a functional fragment or variant thereof, and a first antibody heavy chain constant region; and

(b) a second subunit comprising the antigen-binding site that binds PD-L1, an interleukin-15 receptor alpha (IL-15R α) polypeptide or functional fragment or variant thereof, and a second antibody heavy chain constant region.

32. The bifunctional protein of any one of claims 27-31, wherein the IL-15 polypeptide comprises an amino acids 50-162 of SEQ ID NO:93 or a functional fragment or variant thereof.

33. The bifunctional protein of any one of claims 27-32, wherein the IL-15R α polypeptide comprises an amino acids 31-97 of SEQ ID NO:94 or a functional fragment or variant thereof.

34. The bifunctional protein of any one of claims 27-33, wherein the first subunit comprises an amino acid sequence at least 90% identical to SEQ ID NO:186 and the second subunit comprises an amino acid sequence at least 90% identical to SEQ ID NO:187; optionally wherein the first subunit further comprises an amino acid sequence at least 90% identical to SEQ ID NO:181.

35. The bifunctional protein of claim 34, wherein the first subunit comprises an amino acid sequence of SEQ ID NO:186 and the second subunit comprises an amino acid sequence of SEQ ID NO:187; optionally wherein the first subunit further comprises an amino acid sequence of SEQ ID NO:181.
36. The bifunctional protein of any one of claims 27-33, wherein the first subunit comprising an amino acid sequence at least 90% identical to SEQ ID NO:188 and the second subunit comprising an amino acid sequence at least 90% identical to SEQ ID NO:189.
37. The bifunctional protein of claim 36, wherein the first subunit comprises or consists of the amino acid sequence of SEQ ID NO:188, and the second subunit comprises or consists of the amino acid sequence of SEQ ID NO:189.
38. The bifunctional protein of any one of claims 27-30, wherein the engineered IL-2 polypeptide comprises:
- (a) an IL-2 receptor α (IL-2R α) binding region 1 comprising, relative to wild-type IL-2, one or more mutations at one or more positions selected from:
 - a mutation at position K35 selected from K35G, K35L, K35S, K35V, K35D, K35E, and K35C;
 - a mutation at position R38 selected from R38V, R38D, R38E, R38S, R38I, R38A, R38Y, R38G, R38C, and R38N;
 - a mutation at position F42 selected from F42A, F42R, F42G, F42I, F42L, F42P and F42H; and
 - a mutation at position Y45 selected from Y45S, Y45P, Y45A, Y45V, Y45C, Y45T, and Y45F, and/or
 - (b) an IL-2 receptor β (IL-2R β) binding region 2 motif comprising:

$X_1-X_2-X_3-D-X_4-X_5-X_6-N-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}$ (SEQ ID NO:95),
- wherein:
- X_1 is selected from C, T, G, W, I, S, E, and K;
 - X_2 is selected from Y, P, V, W, L, A, and G;

X₃ is selected from S, T, Q, G, M, E, R, and K;
X₄ is selected from A, V, S, and T;
X₅ is selected from I, L, T, and V;
X₆ is selected from S, T, E, D, and R;
X₇ is selected from I, A, M, and V;
X₈ is selected from S, T, N, Q, I, G, E, K, and R;
X₉ is selected from V, L, and I;
X₁₀ is selected from N, T, I, and L;
X₁₁ is selected from V, A, and I;
X₁₂ is selected from and Q, L, G, K, and R; and
X₁₃ is selected from A, D, and E.

39. The bifunctional protein of claim 38, wherein the engineered IL-2 polypeptide comprises:

- (a) an IL-2R α binding region 1 comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from SEQ ID NOS:124-147; and/or
- (b) an IL-2R β binding region 2 motif comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from SEQ ID NOS:96-123.

40. The bifunctional protein of any one of claims 38-39, wherein:

- (a) the IL-2R α binding region 1 comprises an amino acid sequence selected from SEQ ID NOS:124-147; and/or
- (b) the IL-2R β binding region 2 motif comprises an amino acid sequence selected from SEQ ID NOS:96-123.

41. The bifunctional protein of any one of claims 27-30, comprising an amino acid sequence at least 90% identical to an amino acid sequence selected from SEQ ID NOS:160-164.

42. The bifunctional protein of any one of claims 41, comprising an amino acid sequence selected from SEQ ID NOS:160-164.
43. The bifunctional protein of any one of claims 27-30 and 38-40, comprising:
- (a) a first subunit comprising the antigen-binding site that binds PD-L1 and a first antibody heavy chain constant region; and
 - (b) a second subunit comprising a wild-type interleukin-2 (IL-2) or an engineered IL-2 polypeptide or a functional fragment or variant thereof and a second antibody heavy chain constant region.
44. The bifunctional protein of claim 43, wherein the first subunit comprises an amino acid sequence at least 90% identical to SEQ ID NO:176 and the second subunit comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from SEQ ID NOS:165-175.
45. The bifunctional protein of any one of claims 43-44, wherein the first subunit comprises an amino acid sequence of SEQ ID NO:176 and the second subunit comprises an amino acid sequence selected from SEQ ID NOS:165-175.
46. The bifunctional protein of claim 43, wherein the first subunit comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from SEQ ID NOS:177-180 and the second subunit comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from SEQ ID NOS:165-175, optionally wherein the first subunit further comprises an amino acid sequence at least 90% identical to SEQ ID NO:181.
47. The bifunctional protein of claim 46, wherein the first subunit comprises an amino acid sequence selected from SEQ ID NOS:177-180 and the second subunit comprises an amino acid sequence selected from SEQ ID NOS:165-175, optionally wherein the first subunit further comprises an amino acid sequence of SEQ ID NO:181.

48. The bifunctional protein of claim 46 or 47, wherein the first subunit comprises the amino acid sequence of SEQ ID NO:177 and SEQ ID NO:181, and the second subunit comprises the amino acid sequence of SEQ ID NO:171.
49. The bifunctional protein of any one of claims 27-30 and 38-40, comprising:
- (a) a first subunit comprising the antigen-binding site that binds PD-L1 and a first antibody heavy chain constant region; and
 - (b) a second subunit comprising the antigen-binding site that binds PD-L1, a wild-type interleukin-2 (IL-2) or an engineered IL-2 polypeptide or a functional fragment or variant thereof, and a second antibody heavy chain constant region.
50. The bifunctional protein of claim 49, wherein the first subunit comprises an amino acid sequence at least 90% identical to SEQ ID NO:182 and the second subunit comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from SEQ ID NOS:183-185; optionally wherein the first and the second subunit, each independently, further comprises an amino acid sequence at least 90% identical to SEQ ID NO:181.
51. The bifunctional protein of any one of claims 49-50, wherein the first subunit comprises an amino acid sequence of SEQ ID NO:182 and the second subunit comprises an amino acid sequence selected from SEQ ID NOS:183-185; optionally wherein the first and the second subunit, each independently, further comprises an amino acid sequence of SEQ ID NO:181.
52. The bifunctional protein of any one of claims 28-51, wherein the antibody heavy chain constant region is human IgG heavy chain constant region.
53. The bifunctional protein of any one of claims 28-52, wherein the antibody heavy chain constant region is human IgG1 heavy chain constant region.

54. The bifunctional protein of any one of claims 28-53, wherein the antibody heavy chain constant region comprises an amino acid sequence at least 90% identical to SEQ ID NO:90.
55. The bifunctional protein of any one of claims 28-54, wherein the antibody heavy chain constant region comprises, relative to SEQ ID NO:90, one or more mutations selected from L234A, L235A, P329G, Y349C, S354C, T366S, T366W, L368A, F405K, K409A and Y407V, numbered according to the EU numbering system.
56. The bifunctional protein of any one of claims 31-55, wherein one of the first and the second antibody heavy chain constant regions comprises, relative to SEQ ID NO:90, one or more mutations selected from S354C, T366W and K409A; and the other antibody heavy chain constant region comprises, relative to SEQ ID NO:90, one or more mutations selected from Y349C, T366S, L368A, F405K and Y407V, numbered according to the EU numbering system.
57. The bifunctional protein of any one of claims 31-56, wherein one of the first and the second antibody heavy chain constant regions comprises, relative to SEQ ID NO:90, mutations S354C, T366W and K409A; and the other antibody heavy chain constant region comprises, relative to SEQ ID NO:90, mutations Y349C, T366S, L368A, F405K and Y407V, numbered according to the EU numbering system.
58. The bifunctional protein of any one of claims 31-57, wherein one of the first and the second antibody heavy chain constant regions comprises an amino acid sequence of SEQ ID NO:91 and the other antibody heavy chain constant region comprises an amino acid sequence of SEQ ID NO:92.
59. The bifunctional protein of any one of claims 27-58, wherein the bifunctional protein binds human PD-L1 with a K_D of less than about 1 nM or with a comparable or lower K_D as compared to the comprised antigen-binding site that binds PD-L1, as measured by SPR.

60. The bifunctional protein of any one of claims 27-59, wherein the bifunctional protein binds PD-L1 with a K_D of less than about 0.5 nM or less than about 0.1 nM, as measured by SPR
61. The bifunctional protein of any one of claims 27-60, wherein the bifunctional protein binds PD-L1 with a EC_{50} of less than about 0.4 nM, as measured by ELISA.
62. The bifunctional protein of any one of claims 27-61, wherein the bifunctional protein competes with PD-1 for binding PD-L1 or inhibits PD-L1 binding to PD-1.
63. The bifunctional protein of any one of claims 27-62 wherein the bifunctional protein inhibits PD-L1 binding to PD-1.
64. The bifunctional protein of any one of claims 27-30 and 38-63, wherein the bifunctional protein binds IL-2R β with a K_D of less than about 100 nM, less than about 80 nM, less than about 50 nM, less than about 10 nM, or less than about 5 nM, as measured by SPR.
65. The bifunctional protein of any one of claims 27-30 and 38-64, wherein the bifunctional protein binds IL-2R β with a K_D of less than about 65 nM or less than about 50 nM, as measured by SPR.
66. The bifunctional protein of any one of claims 27-30 and 38-65, wherein the bifunctional protein binds IL-2R α with a K_D of less than about 40 nM, as measured by SPR.
67. The bifunctional protein of any one of claims 27-30 and 38-66, wherein the bifunctional protein binds IL-2R α with a EC_{50} of less than about 1 nM, as measured by ELISA.
68. The bifunctional protein of any one of claims 27-30 and 38-67, wherein the bifunctional protein binds IL-2R β with a EC_{50} of less than about 5 nM, less than

about 2.5 nM, less than about 1.5 nM, less than about 1 nM, or less than about 0.6 nM, as measured by ELISA.

69. The bifunctional protein of any one of claims 27-68, wherein the bifunctional protein induces p-STAT5 expression in immune cells.
70. The bifunctional protein of any one of claims 27-69, wherein the bifunctional protein induces p-STAT5 expression in immune cells with EC₅₀ of less than about 1 nM, less than about 0.6 nM, or less than about 0.1 nM, as measured in isolated human peripheral blood mononuclear cells (PBMCs), and wherein the immune cells are T cells, NK cells, or Tregs.
71. The bifunctional protein of any one of claims 27-70, wherein the bifunctional protein induces p-STAT5 expression in immune cells with EC₅₀ of less than about 0.5 nM or less than about 0.1 nM, as measured in isolated PBMCs, and wherein the immune cells are T cells, NK cells, or Tregs.
72. The bifunctional protein of any one of claims 27-71, wherein the bifunctional protein induces p-STAT5 expression in immune cells as measured in mouse splenocytes, and wherein the immune cells are T cells, NK cells, or Tregs.
73. The bifunctional protein of any one of claims 27-72, wherein the bifunctional protein inhibits tumor growth *in vivo*.
74. The bifunctional protein of any one of claims 27-73, wherein the bifunctional protein induces immune cell proliferation *in vivo*.
75. The bifunctional protein of claim 74, wherein the immune cell is T cell or NK cell.
76. The bifunctional protein of claim 75, wherein the T cell is CD8⁺ T cell.
77. An antibody, comprising a VH and VL, wherein the VH comprises the polypeptide sequence of SEQ ID NO:87 and the VL comprises the polypeptide sequence of SEQ ID NO:88.

78. The antibody of claim 77, wherein the antibody comprises the polypeptide sequences of SEQ ID NO:177 and SEQ ID NO:181.
79. An isolated polynucleotide encoding an antigen-binding site of any one of claims 1-10, a protein of any one of claims 11-26, a bifunctional protein of any one of claims 27-76, or an antibody of claim 77 or 78.
80. An expression vector comprising the polynucleotide of claim 79.
81. A modified cell comprising the isolated polynucleotide of claim 79 or the expression vector of claim 80.
82. A pharmaceutical composition comprising ~~the~~ an antigen-binding site of any one claims of claims 1-10, a protein of any one of claims 11-26, a bifunctional protein of any one of claims 27-76, or an antibody of claim 77 or 78, and a pharmaceutically acceptable carrier.
83. An antigen-binding site of any one ~~claims~~ of claims 1-10, a protein of any one of claims 11-26, a bifunctional protein of any one of claims 27-76, or an antibody of claim 77 or 78, for use in ~~a method of~~ modulating an immune response in a subject in need thereof.
84. The antigen-binding site, protein, bifunctional protein, or antibody~~method for use according to~~ of claim 83, wherein modulating the immune response comprises at least one of enhancing T cell activity or enhancing NK cell activity.
85. An antigen-binding site of any one ~~claims~~ of claims 1-10, a protein of any one of claims 11-26, a bifunctional protein of any one of claims 27-76, or an antibody of claim 77 or 78, for use in ~~a method of~~ treating a disease in a subject in need thereof.
86. The antigen-binding site of any one ~~claims~~ of claims 1-10, a protein of any one of claims 11-26, a bifunctional protein of any one of claims 27-76, or an antibody of claim 77 or 78, for use according to claim 85, wherein the disease comprises cancer.

87. The antigen-binding site of any one ~~claims~~ of claims 1-10, a protein of any one of claims 11-26, a bifunctional protein of any one of claims 27-76, or an antibody of claim 77 or 78, for use according to claim 86, wherein the cancer comprises breast cancer, pancreatic cancer, lung cancer, glioblastoma, renal cell carcinoma, or melanoma.
88. The antigen-binding site of any one ~~claims~~ of claims 1-10, a protein of any one of claims 11-26, a bifunctional protein of any one of claims 27-76, or an antibody of claim 77 or 78, for use according to any of claims 83-87, wherein the subject is to be treated with an additional therapeutic agent.
89. Use of ~~A method of modulating an immune response in a subject in need thereof, the method comprising administering to the subject~~ a therapeutically effective amount of an antigen-binding site of any one ~~claims~~ of claims 1-10, a protein of any one of claims 11-26, a bifunctional protein of any one of claims 27-76, or an antibody of claim 77 or 78, or a pharmaceutical composition thereof in the manufacture of a medicament for modulating an immune response in a subject in need thereof.
90. The ~~method~~ use of claim 89, wherein modulating the immune response comprises at least one of: enhancing effector T cell activity, enhancing NK cell activity, and suppressing regulatory T cell activity.
91. Use of ~~A method of treating a disease in a subject in need thereof, the method comprising administering to the subject~~ a therapeutically effective amount of an antigen-binding site of any one ~~claims~~ of claims 1-10, a protein of any one of claims 11-26, a bifunctional protein of any one of claims 27-76, or an antibody of claim 77 or 78, or a pharmaceutical composition thereof, in the manufacture of a medicament for treating a disease in a subject in need thereof.
92. The ~~method~~ use of claim 91, wherein the disease is cancer.

93. The ~~method~~use of claim 92, wherein the cancer comprises breast cancer, pancreatic cancer, lung cancer, glioblastoma, renal cell carcinoma, or melanoma.