

immunotherapeutic agents with increased potency and efficacy for use in the treatment of cancer.

In one aspect, the present disclosure provides a multispecific antigen-binding construct comprising at least two antigen-binding arms, wherein: a first arm binds PD-1 and comprises a heavy chain variable region and a light chain variable region, the heavy chain variable region of the first arm comprising a CDRH1 of SEQ ID NO: 77, a CDRH2 of SEQ ID NO: 71, and a CDRH3 of SEQ ID NO: 75, and a second arm binds a PD-1 ligand and comprises a heavy chain variable region and a light chain variable region, the heavy chain variable region of the second arm comprising a CDRH1 of SEQ ID NO: 1, a CDRH2 of SEQ ID NO: 7, and a CDRH3 of SEQ ID NO: 8; wherein the light chain variable region of the first arm and the light chain variable region of the second arm each comprise a CDRL1 of SEQ ID NO: 9, a CDRL2 of SEQ ID NO: 5, and a CDRL3 of SEQ ID NO: 10; and wherein the multispecific antigen-binding construct blocks the interaction of PD-1 and the PD-1 ligand.

In some examples, any of the multispecific antigen-binding constructs disclosed herein binds to at least two different receptors or epitopes (*e.g.*, PD-1 and PD-L1), wherein the two different receptors or epitopes bound by the multispecific antigen-binding construct are expressed on the surface of the same cell. For example, in some examples, the multispecific antigen-binding construct simultaneously binds to PD-1 and PD-L1, wherein the PD-1 and PD-L1 are expressed on the surface of the same cell. In some examples, any of the multispecific antigen-binding constructs disclosed herein binds to at least two different receptors or epitopes (*e.g.*, PD-1 and PD-L1), wherein the two different receptors or epitopes bound by the multispecific antigen-binding construct are expressed on the surface of two different cells. For example, in some examples, the multispecific antigen-binding construct simultaneously binds to PD-1 expressed on the surface of a first cell and to a PD-1 ligand, *e.g.*, PD-L1 or PD-L2, expressed on the surface of a second cell.

In some examples, the disclosure provides for a multispecific antigen-binding construct comprising at least two antigen-binding arms, wherein a first arm binds PD-1 expressed by an immune cell, and a second arm binds a PD-1 ligand which is expressed by a second cell, wherein the multispecific antigen-binding construct blocks the interaction of PD-1 and PD-1 ligand. In some examples, the PD-1 ligand is PD-L2. In some examples, the PD-1 ligand is PD-L1. In some examples, the immune cell is a T cell. In some examples, the T cell is a CD8⁺ T cell. In some examples, the immune cell is a natural killer (NK) cell. In

some examples, the immune cell is a macrophage. In some examples, the second cell is a second immune cell. In some examples, the second immune cell is any one or more of a T cell, a B cell, a macrophage, a myeloid-derived suppressor cell, a dendritic cell, or a mesenchymal stromal cell. In some examples, the second immune cell is a regulatory T cell.

- 5 In some examples, the second cell is a tumor cell. In some examples, the tumor cell is selected from the group consisting of a hematological cancer, a lymphoma, a myeloma, a leukemia, a neurological cancer, melanoma, breast cancer, a prostate cancer, a colorectal cancer, lung cancer, head and neck cancer, a gastrointestinal cancer, liver cancer, pancreatic cancer, a genitourinary cancer, a bone cancer, renal cancer, and a vascular cancer. In some
- 10 examples, both arms have a K_D of at least 1×10^{-7} M, at least 1×10^{-8} M, at least 1×10^{-9} M, or at least 1×10^{-10} M. In some examples, the binding of one arm to its target does not block the binding of the other arm to its target. In some examples, the first arm and second arm bind to their respective

targets and both arms remain bound concurrently. In some examples, binding of the first arm and the second arm to their respective targets can bridge the immune cell and the second cell together. In some examples, the bridging of the immune cell and the second cell is determined by flow cytometry. In some examples, the first arm is an antagonist of PD-1.

5 In some examples of the multispecific antigen-binding constructs, the first arm binds to PD-1 and comprises: (a) a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 70 (FTFX₁X₂YAX₃X₄, wherein X₁=S, R, G, or N; X₂=D, S, N, A, R, or G; X₃=M or L; X₄=S, L, or N); (ii) a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA); and (iii) a CDRH3 comprising SEQ ID NO: 72 (ARGLDFIVGX₅TGNDY, wherein X₅=A, Y, or R); and (b) a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the CDRH1 of the first arm comprises SEQ ID NO: 73 (FTFSDYAMS), CDRH2 of the first arm comprises SEQ ID NO: 71 (SAISNSGTYTYYA) and CDRH3 of the first arm comprises SEQ ID NO: 74 (ARGLDFIVGATGNDY). In some examples, the CDRH1 of the first arm comprises SEQ ID NO: 73 (FTFSDYAMS), CDRH2 of the first arm comprises SEQ ID NO: 71 (SAISNSGTYTYYA) and CDRH3 of the first arm comprises SEQ ID NO: 75 (ARGLDFIVGYTGNDY). In some examples, the CDRH1 of the first arm comprises SEQ ID NO: 76 (FTFSSYAMS), CDRH2 of the first arm comprises SEQ ID NO: 71 (SAISNSGTYTYYA) and CDRH3 of the first arm comprises SEQ ID NO: 75 (ARGLDFIVGYTGNDY). In some examples, the CDRH1 of the first arm comprises SEQ ID NO: 77 (FTFSSYAML), CDRH2 of the first arm comprises SEQ ID NO: 71 (SAISNSGTYTYYA) and CDRH3 of the first arm comprises SEQ ID NO: 75 (ARGLDFIVGYTGNDY). In some examples, the CDRH1 of the first arm comprises SEQ ID NO: 78 (FTFSNYALS), CDRH2 of the first arm comprises SEQ ID NO: 71 (SAISNSGTYTYYA) and CDRH3 of the first arm comprises SEQ ID NO: 75 (ARGLDFIVGYTGNDY). In some examples, the CDRH1 of the first arm comprises SEQ ID NO: 79 (FTFSAYAMN), CDRH2 of the first arm comprises SEQ ID NO: 71 (SAISNSGTYTYYA) and CDRH3 of the first arm comprises SEQ ID NO: 75 (ARGLDFIVGYTGNDY). In some examples, the CDRH1 of the first arm comprises SEQ ID NO: 80 (FTFRSYAMS), CDRH2 of the first arm comprises SEQ ID NO: 71 (SAISNSGTYTYYA) and CDRH3 of the first arm comprises SEQ ID NO: 75 (ARGLDFIVGYTGNDY). In some examples, the CDRH1 of the first arm comprises

SEQ ID NO: 81 (FTFGRYAMS), CDRH2 of the first arm comprises SEQ ID NO: 71 (SAISNSGTYTYYA) and CDRH3 of the first arm comprises SEQ ID NO: 75 (ARGLDFIVGYTGNDY). In some examples, the CDRH1 of the first arm comprises SEQ ID NO: 82 (FTFNSYAMS), CDRH2 of the first arm comprises SEQ ID NO: 71 (SAISNSGTYTYYA) and CDRH3 of the first arm comprises SEQ ID NO: 75 (ARGLDFIVGYTGNDY). In some examples, the CDRH1 of the first arm comprises SEQ ID NO: 83 (FTFSNYAMS), CDRH2 of the first arm comprises SEQ ID NO: 71 (SAISNSGTYTYYA) and CDRH3 of the first arm comprises SEQ ID NO: 74 (ARGLDFIVGATGNDY). In some examples, the CDRH1 of the first arm comprises SEQ ID NO: 84 (FTFSGYAMS), CDRH2 of the first arm comprises SEQ ID NO: 71 (SAISNSGTYTYYA) and CDRH3 of the first arm comprises SEQ ID NO: 85 (ARGLDFIVGRTGNDY). In some examples, the CDRH1 of the first arm comprises SEQ ID NO: 86 (FTFSSYAMN), CDRH2 of the first arm comprises SEQ ID NO: 71 (SAISNSGTYTYYA) and CDRH3 of the first arm comprises SEQ ID NO: 85 (ARGLDFIVGRTGNDY). In some examples, the CDRH1 of the first arm comprises SEQ ID NO: 80 (FTFRSYAMS), CDRH2 of the first arm comprises SEQ ID NO: 71 (SAISNSGTYTYYA) and CDRH3 of the first arm comprises SEQ ID NO: 85 (ARGLDFIVGRTGNDY).

In some examples, the CDRL1 of the first arm comprises SEQ ID NO: 9 (RASQSISSYLN), CDRL2 of the first arm comprises SEQ ID NO: 5 (AASSLQS) and CDRL3 of the first arm comprises SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 87. In some examples, the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 88. In some examples, the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 89. In some examples, the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 90. In some examples, the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 91. In some examples, the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 92. In some examples, the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 93. In some examples, the heavy chain variable region of the first arm comprises an amino

acid sequence that is at least 90% identical to SEQ ID NO: 94. In some examples, the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 95. In some examples, the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 96. In some examples, the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 97. In some examples, the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 98. In some examples, the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 99. In some examples, the light chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 59.

In some examples, the second arm is an antagonist of the PD-1 ligand. In some examples, the second arm is an antagonist of PD-L2. In some examples, the second arm is an antagonist of PD-L1. In some examples, the second arm binds to PD-L1 and comprises: a. a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN); (ii) a CDRH2 comprising SEQ ID NO: 2 (GGIIPX₁X₂GX₃ATYA, wherein X₁ is V or I; X₂ is F, L, or V; and X₃ is T or A); and (iii) a CDRH3 comprising SEQ ID NO: 3 (ARLKX₁ELKDAFDI, wherein X₁ is G, F, or N); and b. a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 4 (RASQX₁ISSYLN, wherein X₁ is S, W, or Q); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 6 (X₁QSYSTPLT, wherein X₁ is Q or F).

In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 7 (GGIIPILGAATYA) and CDRH3 of the second arm comprises SEQ ID NO: 8 (ARLKGELKDAFDI). In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 7 (GGIIPILGAATYA), CDRH3 of the second arm comprises SEQ ID NO: 8 (ARLKGELKDAFDI); CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQSISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 11 (GGIIPVFGTATYA), CDRH3 of the second arm comprises SEQ ID NO: 8 (ARLKGELKDAFDI); CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQSISSYLN); CDRL2 of the second arm

comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10 (QSYSTPLT). In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 11 (GGIIPVFGTATYA) and CDRH3 of the second arm comprises SEQ ID NO: 8

5 (ARLKGELKDAFDI). In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 11 (GGIIPVFGTATYA), CDRH3 of the second arm comprises SEQ ID NO: 8 (ARLKGELKDAFDI); CDRL1 of the second arm comprises SEQ ID NO: 12 (RASQWISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and
10 CDRL3 of the second arm comprises SEQ ID NO: 10 (QSYSTPLT). In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 11 (GGIIPVFGTATYA), CDRH3 of the second arm comprises SEQ ID NO: 8 (ARLKGELKDAFDI); CDRL1 of the second arm comprises SEQ ID NO: 13 (RASQISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5
15 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10 (QSYSTPLT). In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 11 (GGIIPVFGTATYA), CDRH3 of the second arm comprises SEQ ID NO: 8 (ARLKGELKDAFDI); CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQISSYLN); CDRL2 of the second arm comprises SEQ ID
20 NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10 (QSYSTPLT).

In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 15 (GGIIPIFGIANYA) and CDRH3 of the second arm comprises SEQ ID NO: 8 (ARLKGELKDAFDI). In some
25 examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 15 (GGIIPIFGIANYA), CDRH3 of the second arm comprises SEQ ID NO: 8 (ARLKGELKDAFDI); CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10 (QSYSTPLT).

30 In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 16 (GGIIPNFGTATYA) and CDRH3 of the second arm comprises SEQ ID NO: 17 (ARLKGELKGAGDI). In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN),

CDRH2 of the second arm comprises SEQ ID NO: 16 (GGIIPNFGTATYA), CDRH3 of the second arm comprises SEQ ID NO: 17 (ARLKGELKGAGDI); CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQSISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10

5 (QQSYSTPLT).

In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 11 (GGIIPVFGTATYA), and CDRH3 of the second arm comprises SEQ ID NO: 18 (ARLKFELKDAFDI). In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2
10 of the second arm comprises SEQ ID NO: 11 (GGIIPVFGTATYA), CDRH3 of the second arm comprises SEQ ID NO: 18 (ARLKFELKDAFDI), CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQSISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1
15 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 11 (GGIIPVFGTATYA), and CDRH3 of the second arm comprises SEQ ID NO: 19 (ARLKGELKDAFDE). In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 11 (GGIIPVFGTATYA), CDRH3 of the second arm comprises SEQ ID NO: 19 (ARLKGELKDAFDE), CDRL1 of the second arm comprises
20 SEQ ID NO: 9 (RASQSISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 11 (GGIIPVFGTATYA), and CDRH3 of the second arm comprises SEQ ID NO: 20 (ARLKNELKDAFDI). In some
25 examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 11 (GGIIPVFGTATYA), CDRH3 of the second arm comprises SEQ ID NO: 20 (ARLKNELKDAFDI), CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQSISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10 (QQSYSTPLT).

30 In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 21

(GGVIPFLGTANYA), and CDRH3 of the second arm comprises SEQ ID NO: 22 (ARLKGILKDALDI). In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 21 (GGVIPFLGTANYA), CDRH3 of the second arm comprises SEQ ID NO: 22 (ARLKGILKDALDI), CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQSISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 29 (GRIPIFGTADYA), and CDRH3 of the second arm comprises SEQ ID NO: 8 (ARLKGELKDAFDI). In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 29 (GRIPIFGTADYA), CDRH3 of the second arm comprises SEQ ID NO: 8 (ARLKGELKDAFDI), CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQSISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 31 (GGIIPILGTATYA), and CDRH3 of the second arm comprises SEQ ID NO: 32 (ARRKGELKDAFDI). In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 31 (GGIIPILGTATYA), CDRH3 of the second arm comprises SEQ ID NO: 32 (ARRKGELKDAFDI), CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQSISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 33 (GGIPIVATANYA), and CDRH3 of the second arm comprises SEQ ID NO: 32 (ARRKGELKDAFDI). In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 33 (GGIPIVATANYA), CDRH3 of the second arm comprises SEQ ID NO: 32 (ARRKGELKDAFDI), CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQSISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 34 (GGIPIFGKATYA), and CDRH3 of the second arm comprises SEQ ID NO: 32 (ARRKGELKDAFDI). In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 34 (GGIPIFGKATYA), CDRH3 of the second arm comprises SEQ ID NO: 32 (ARRKGELKDAFDI), CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQSISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 11 (GGIIPVFGTATYA), CDRH3 of the second arm comprises SEQ ID NO: 8 (ARLKGELKDAFDI); CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQSISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 38 (FQSYSTPLT).

In some examples, the CDRH1 of the second arm comprises SEQ ID NO: 1 (GTFSSYAIN), CDRH2 of the second arm comprises SEQ ID NO: 11 (GGIIPVFGTATYA), CDRH3 of the second arm comprises SEQ ID NO: 8 (ARLKGELKDAFDI); CDRL1 of the second arm comprises SEQ ID NO: 9 (RASQSISSYLN); CDRL2 of the second arm comprises SEQ ID NO: 5 (AASSLQS); and CDRL3 of the second arm comprises SEQ ID NO: 39 (QQSYSTILT).

In some examples, the second arm comprises: a. a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 14 (GTFSSYAFS), (ii) a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA) and (iii) a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI); and b. a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the second arm comprises a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 23 (GTFSSYAIS), (ii) a CDRH2 comprising SEQ ID NO: 24 (GGIPIVGIANYA), and (iii) a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI). In some examples, the second arm comprises a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the second arm comprises a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), (ii) a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), and (iii) a CDRH3 comprising SEQ ID NO: 25 (ARLKGEFKDAFDI). In some examples, the second arm comprises a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the second arm comprises a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), (ii) a CDRH2 comprising SEQ ID NO: 26 (GRIIPLFGTAHYA), and (iii) a CDRH3 comprising SEQ ID NO: 8 (ARLKGEFKDAFDI). In some examples, the second arm comprises a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the second arm comprises a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), (ii) a CDRH2 comprising SEQ ID NO: 27 (GRINPILGTANYA), and (iii) a CDRH3 comprising SEQ ID NO: 28 (ARLKGEFKDAFSI). In some examples, the second arm comprises a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the second arm comprises a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), (ii) a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), and (iii) a CDRH3 comprising SEQ ID NO: 30 (ARLKGEFKCAFDI). In some examples, the second arm comprises a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the second arm comprises a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 122 (GTKSSY AIS), (ii) CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), and (iii) a CDRH3 comprising SEQ ID NO: 30 (ARLKGEFKCAFDI). In some examples, the second arm further comprises a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 9

(RASQSISSYLN); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the second arm comprises a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 36 (GPFRSHAVS), (ii) a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), and (iii) a CDRH3 comprising SEQ ID NO: 37 (ARLKSELKDAFDI). In some examples, the second arm comprises a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT).

In some examples, the second arm that binds PD-L1 comprises a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to any one of SEQ ID NOs: 35, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, or 58 and a light chain variable region comprising an amino acid sequence that is at least 90% identical to any one of SEQ ID NOs: 59, 60, 61, 62, or 63.

In some examples, the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 35 (QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAINWVRQAPGQGLEWMGGIIPVFGTATYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARLKGELKDAFDIWGGTMVTVSS). In some examples, the heavy chain variable region of the second arm

comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 40 (QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAFSWVRQAPGQGLEWMGGIIPVFGTATYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARLKGELKDAFDIWGGTLVTVSS). In some examples, the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 41

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAINWVRQAPGQGLEWMGGIIPFGIANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARLKGELKDAFDIWGGQTLVTVSS). In some examples, the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 42

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAINWVRQAPGQGLEWMGGIIPNFGTATYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARLKGELKGAGDIWGGTLVTVSS). In some examples, the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 43

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAINWVRQAPGQGLEWMGGIIPVFGTATYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARLKFELKDAFDIWGGQ

TLVTVSS). In some examples, the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 44

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAINWVRQAPGQGLEWMGGIIPVFG
TATYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCARLKGELKDAFDEWGQ

5 GTLVTVSS). In some examples, the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 45

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAINWVRQAPGQGLEWMGGIIPVFG
TATYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCARLKGELKDAFDIWGQ

10 GTLVTAST). In some examples, the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 46

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAINWVRQAPGQGLEWMGGIIPVFG
TATYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCARLKNELKDAFDIWGQ

GTLVTVSS). In some examples, the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 47

15 (QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAINWVRQAPGQGLEWMGGVIPFL
GTANYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCARLKGILKDALDIWG

QGTTLVTVSS). In some examples, the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 48

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQDLEWMGGIIPVGI
20 ANYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCARLKGELKDAFDIWGQ

TLVTVSS). In some examples, the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 49

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGGIIPVFG
TATYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCARLKGFEKDAFDIWGQ

25 GTLVTVSS). In some examples, the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 50

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGRIPFLG
TAHYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCARLKGELKDAFDIWGQ

GTLVTVSS). In some examples, the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 51

30 (QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGRINPILG
TANYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCARLKGELKDAFSIWGQ

GTLVTVSS). In some examples, the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 52

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAINWVRQAPGQGLEWMGRIIPFG
TADYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARLKGELKDAFDIWGQ
GTLVTVSS). In some examples, the heavy chain variable region of the second arm
comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 53

5 (QVQLVQSGAEVKKPGSSVKVSCASGGKFSSYAIWVRQAPGQGLEWMGGIIPVFG
TATYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARLKGELKCAFDIWGQ
GTLVTVSS). In some examples, the heavy chain variable region of the second arm
comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 54

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAINWVRQAPGQGLEWMGGIIPILG
10 TATYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARRKGELKDAFDIWGQ
GTLVTVSS). In some examples, the heavy chain variable region of the second arm
comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 55

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAINWVRQAPGQGLEWMGGIIPILG
AATYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARLKGELKDAFDIWGQ
15 GTLVTVSS). In some examples, the heavy chain variable region of the second arm
comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 56

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAINWVRQAPGQGLEWMGGIIPIVA
TANYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARRKGELKDAFDIWGQ
GTLVTVSS). In some examples, the heavy chain variable region of the second arm
20 comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 57

(QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAINWVRQAPGQGLEWMGGIIPIFG
KATYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARRKGELKDAFDIWGQ
GTLVTVSS). In some examples, the heavy chain variable region of the second arm
comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 58

25 (QVQLVQSGAEVKKPGSSVKVSCASGGPFRSHAVSWVRQAPGQGLEWMGGIIPVF
GTATYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARLKSELKDAFDIWGQ
GTLVTVSS). In some examples, the light chain variable region of the second arm comprises
an amino acid sequence that is at least 90% identical to SEQ ID NO: 59

(DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGV
30 PSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPLTFGGGTKVEIK). In some
examples, the light chain variable region of the second arm comprises an amino acid
sequence that is at least 90% identical to SEQ ID NO: 60

(DIQMTQSPSSLSASVGDRVTITCRASQWISSYLNWYQQKPGKAPKLLIYAASSLQSG
VPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPLTFGGGTKVEIK). In some

examples, the light chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 61

(DIQMTQSPSSLSASVGDRVTITCRASQQISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFGSGSGTDFLTITSSLPEDFATYYCQQSYSTPLTFGGGTKVEIK). In some

examples, the light chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 62

(DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFGSGSGTDFLTITSSLPEDFATYYCFQSYSTPLTFGGGTKVEIK). In some

examples, the light chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 63

(DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFGSGSGTDFLTITSSLPEDFATYYCQQSYSTILTFGGGTKVEIK).

In some examples of any of the examples described herein, the construct is a bispecific antibody. In some examples, the bispecific antibody is an antagonist of both PD-1 and PD-1 ligand. In some examples, the construct comprises a common light chain. In some examples, one or both of the arms is an aptamer. In some examples, one or both of the arms is a protein other than an antibody. In some examples, the construct comprises at least two bispecific antibodies. In some examples, one of the at least two bispecific antibodies is monovalent for PD-1. In some examples, one of the at least two bispecific antibodies is monovalent for PD-1 ligand. In some examples, at least one of the arms is a bivalent antibody specific for PD-1. In some examples, at least one of the arms is a bivalent antibody specific for PD-L1. In some examples, at least one of the arms is a bivalent antibody specific for PD-1, and at least one of the arms is a bivalent antibody specific for PD-L1. In some examples, at least one of the arms is bivalent for PD-1. In some examples, at least one of the arms is bivalent for PD-L1. In some examples, at least one of the arms is bivalent for PD-1, and at least one of the arms is bivalent for PD-L1. In some examples, the bispecific antibody binds two different epitopes on PD-1. In some examples, the bispecific antibody binds two different epitopes on the PD-1 ligand.

In some examples, any of the multispecific antigen-binding constructs disclosed herein comprises at least two monospecific antibodies. In some examples, at least one of the monospecific antibodies is an anti-PD-1 antibody. In some examples, the anti-PD-1 antibody is a bivalent anti-PD-1 antibody. In some examples, at least one of the monospecific antibodies is an anti-PD-L1 antibody. In some examples, the anti-PD-1

antibody is a bivalent anti-PD-L1 antibody. In some examples, the construct comprises a bivalent anti-PD-1 antibody and a bivalent anti-PD-L1 antibody. In some examples, the construct is a fusion construct in which a polypeptide comprising the variable heavy chain of the anti-PD-1 antibody is fused to a polypeptide comprising the variable heavy chain of the anti-PD-L1 antibody. In some examples, the polypeptide comprising the variable heavy chain of the anti-PD-1 antibody is fused to the polypeptide comprising the variable heavy chain of the anti-PD-L1 antibody by means of a linker. In some examples, the fusion construct comprises a common light chain. In some examples, the N-terminal variable heavy chain of the fusion construct binds to PD-1 in the presence of the common light chain, and the C-terminal variable heavy chain of the fusion construct binds to PD-L1 in the presence of the common light chain. In some examples, the N-terminal variable heavy chain of the fusion construct binds to PD-L1 in the presence of the common light chain, and the C-terminal variable heavy chain of the fusion construct binds to PD-1 in the presence of the common light chain.

In some examples and examples, the disclosure provides for a multispecific antigen-binding construct comprising at least two units of antigen-binding, wherein a first unit of antigen-binding binds PD-1, and a second unit of antigen-binding binds a PD-1 ligand. In some examples, the first unit of antigen-binding binds PD-1 expressed by an immune cell. In some examples, the second unit of antigen-binding binds PD-1 expressed by a second cell. In some examples, the multispecific antigen-binding construct blocks the interaction of PD-1 and a PD-1 ligand, such as PD-L1 or PD-L2. In some examples, the multispecific antigen-binding construct blocks the interaction of PD-1 and a PD-1 ligand, such as PD-L1 or PD-L2. In some examples, the multispecific antigen-binding construct comprises at least two units of antigen-binding that bind PD-1. In some examples, the multispecific antigen-binding construct comprises two units of antigen-binding that bind PD-1. In some examples, the multispecific antigen-binding construct comprises at least two units of antigen-binding that bind a PD-1 ligand, such as PD-L1 or PD-L2. In some examples, the multispecific antigen-binding construct comprises two units of antigen-binding that bind a PD-1 ligand, such as PD-L1 or PD-L2. In some examples, the multispecific antigen-binding construct comprises at least four units of antigen-binding, wherein two units of antigen-binding bind PD-1 and two units of antigen-binding bind a PD-1 ligand, such as PD-L1 or PD-L2. In some examples, the multispecific antigen-binding construct comprises four units of antigen-binding, wherein two units of antigen-binding bind PD-1 and two units of antigen-binding bind a PD-1 ligand, such as PD-L1 or PD-L2. In some

examples, each unit of antigen-binding is capable of binding independently to its cognate antigen, *i.e.*, PD-1 or a PD-1 ligand, such as PD-L1 or PD-L2. In some examples, the multispecific antigen-binding construct promotes loss of PD-1 expression from a cell. In some examples, the loss of PD-1 expression is due to PD-1 shedding. In some examples, the multispecific antigen-binding construct blocks interaction of PD-1 and the PD-1 ligand, such as PD-L1 or PD-L2. In some examples, the multispecific antigen-binding construct comprises a common light chain. For example, at least two units of antigen-binding comprise a common light chain.

In some examples, the first unit of antigen-binding binds PD-1 and comprises:

- (a) a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 70 (FTFX₁X₂YAX₃X₄, wherein X₁=S, R, G, or N; X₂=D, S, N, A, R, or G; X₃= M or L; X₄= S, L, or N); (ii) a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA); and (iii) a CDRH3 comprising SEQ ID NO: 72 (ARGLDFIVGX₅TGNDY, wherein X₅=A, Y, or R); and
- (b) a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT).

In some such examples, the first unit of antigen-binding binds PD-1 and comprises:

- (a) a CDRH1 comprising SEQ ID NO: 73 (FTFSDYAMS), a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA), and a CDRH3 comprising SEQ ID NO: 74 (ARGLDFIVGATGNDY);
- (b) a CDRH1 comprising SEQ ID NO: 73 (FTFSDYAMS), a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA), and a CDRH3 comprising SEQ ID NO: 75 (ARGLDFIVGYTGNDY);
- (c) a CDRH1 comprising SEQ ID NO: 76 (FTFSSYAMS), a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA), and a CDRH3 comprising SEQ ID NO: 75 (ARGLDFIVGYTGNDY);
- (d) a CDRH1 comprising SEQ ID NO: 77 (FTFSSYAML), a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA), and a CDRH3 comprising SEQ ID NO: 75 (ARGLDFIVGYTGNDY);
- (e) a CDRH1 comprising SEQ ID NO: 78 (FTFSNYALS), a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA), and a CDRH3 comprising SEQ ID NO: 75 (ARGLDFIVGYTGNDY);

- (f) a CDRH1 comprising SEQ ID NO: 79 (FTFSAYAMN), a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA), and a CDRH3 comprising SEQ ID NO: 75 (ARGLDFIVGYTGNDY);
- 5 (g) a CDRH1 comprising SEQ ID NO: 80 (FTFRSYAMS), a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA), and a CDRH3 comprising SEQ ID NO: 75 (ARGLDFIVGYTGNDY);
- (h) a CDRH1 comprising SEQ ID NO: 81 (FTFGRYAMS), a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA), and a CDRH3 comprising SEQ ID NO: 75 (ARGLDFIVGYTGNDY);
- 10 (i) a CDRH1 comprising SEQ ID NO: 82 (FTFNSYAMS), a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA), and a CDRH3 comprising SEQ ID NO: 75 (ARGLDFIVGYTGNDY);
- (j) a CDRH1 comprising SEQ ID NO: 83 (FTFSNYAMS), a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA), and a CDRH3 comprising SEQ ID NO: 74 (ARGLDFIVGATGNDY);
- 15 (k) a CDRH1 comprising SEQ ID NO: 84 (FTFSGYAMS), a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA), and a CDRH3 comprising SEQ ID NO: 85 (ARGLDFIVGRTGNDY);
- (l) a CDRH1 comprising SEQ ID NO: 86 (FTFSSYAMN), a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA) and a CDRH3 comprising SEQ ID NO: 85 (ARGLDFIVGRTGNDY); or
- 20 (m) a CDRH1 comprising SEQ ID NO: 80 (FTFRSYAMS), a comprising SEQ ID NO: 71 (SAISNSGTYTYYA), and a CDRH3 comprising SEQ ID NO: 85 (ARGLDFIVGRTGNDY).
- 25 In some examples, the first unit of antigen-binding binds PD-1 and comprises:
- (a) a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 87;
- (b) a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 88;
- 30 (c) a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 89;
- (d) a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 90;

- (e) a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 91;
- (f) a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 92;
- 5 (g) a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 93;
- (h) a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 94;
- 10 (i) a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 95;
- (j) a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 96;
- (k) a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 97;
- 15 (l) a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 98; or
- (m) a heavy chain variable region comprising amino acid sequence that is at least 90% identical to SEQ ID NO: 99.

20 In some examples, the first unit of antigen-binding binds PD-1 and comprises a light chain variable region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 59.

In some examples, the second unit of antigen-binding binds PD-L2. In some examples, the second unit of antigen-binding binds PD-L1. In some examples, the second unit of antigen-binding binds PD-L1 and comprises:

- 25 a. a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN); (ii) a CDRH2 comprising SEQ ID NO: 2 (GGIIPX₁X₂GX₃ATYA, wherein X₁ is V or I; X₂ is F, L, or V; and X₃ is T or A); and (iii) a CDRH3 comprising SEQ ID NO: 3 (ARLKX₁ELKDAFDI, wherein X₁ is G, F, or N); and
- b. a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 4 (RASQX₁ISSYLN, wherein X₁ is S, W, or Q); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 6 (X₁QSYSTPLT, wherein X₁ is Q or F).
- 30

In some such examples, the second unit of antigen-binding binds PD-L1 and comprises:

- (a) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 7 (GGIIPILGAATYA), and a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI);
- 5 (b) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 7 (GGIIPILGAATYA), a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- 10 (c) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- 15 (d) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA) and a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI);
- (e) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI); a CDRL1 comprising SEQ ID NO: 12 (RASQWISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- 20 (f) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI); a CDRL1 comprising SEQ ID NO: 13 (RASQQISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- 25 (g) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- 30 (h) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 15 (GGIIPIFGIANYA), and a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI);

- 5 (i) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 15 (GGIPIFGIANYA), a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (CQQSYSTPLTF);
- (j) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 16 (GGIIPNFGTATYA), and a CDRH3 comprising SEQ ID NO: 17 (ARLKGELKGAGDI);
- 10 (k) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 16 (GGIIPNFGTATYA), a CDRH3 comprising SEQ ID NO: 17 (ARLKGELKGAGDI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- 15 (l) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), and a CDRH3 comprising SEQ ID NO: 18 (ARLKFELKDAFDI);
- (m) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), a CDRH3 comprising SEQ ID NO: 18 (ARLKFELKDAFDI), a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- 20 (n) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), and a CDRH3 comprising SEQ ID NO: 19 (ARLKGELKDAFDE);
- 25 (o) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), a CDRH3 comprising SEQ ID NO: 19 (ARLKGELKDAFDE), a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- 30 (p) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), and a CDRH3 comprising SEQ ID NO: 20 (ARLKNELKDAFDI);
- (q) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), a CDRH3 comprising SEQ ID NO: 20

- (ARLKNELKDAFDI), a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- 5 (r) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 21 (GGVIPFLGTANYA), and a CDRH3 comprising SEQ ID NO: 22 (ARLKGILKDALDI);
- (s) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 21 (GGVIPFLGTANYA), a CDRH3 comprising SEQ ID NO: 22 (ARLKGILKDALDI), a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a
- 10 CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- (t) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising EQ ID NO: 29 (GRIPIFGTADYA), and a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI);
- 15 (u) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 29 (GRIPIFGTADYA), a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI), a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- 20 (v) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 31 (GGIIPILGTATYA), and a CDRH3 comprising SEQ ID NO: 32 (ARRKGELKDAFDI);
- (w) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 31 (GGIIPILGTATYA), a CDRH3 comprising SEQ ID NO: 32 (ARRKGELKDAFDI), a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a
- 25 CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- (x) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 33 (GGIPIVATANYA), and a CDRH3 comprising SEQ ID NO: 32 (ARRKGELKDAFDI);
- 30 (y) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 33 (GGIPIVATANYA), a CDRH3 comprising SEQ ID NO: 32 (ARRKGELKDAFDI), a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a

CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);

(z) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 34 (GGIPIFGKATYA), and a CDRH3 comprising SEQ ID NO: 32 (ARRKGELKDAFDI);

5 (aa) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 34 (GGIPIFGKATYA), a CDRH3 comprising SEQ ID NO: 32 (ARRKGELKDAFDI), a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);

10 (bb) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 38 (FQSYSTPLT);

15 (cc) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 39 (QQSYSTILT);

20 (dd) a CDRH1 comprising SEQ ID NO: 14 (GTFSSYAFS), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA) and a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);

25 (ee) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), a CDRH2 comprising SEQ ID NO: 24 (GGIPIVGIANYA), and a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI);

(ff) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), a CDRH2 comprising SEQ ID NO: 24 (GGIPIVGIANYA), and a CDRH3 comprising SEQ ID NO: 8 (ARLKGELKDAFDI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);

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- (gg) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), and a CDRH3 comprising SEQ ID NO: 25 (ARLKGEFKDAFDI);
- 5 (hh) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), and a CDRH3 comprising SEQ ID NO: 25 (ARLKGEFKDAFDI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- 10 (ii) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), a CDRH2 comprising SEQ ID NO: 26 (GRIIPLFGTAHYA), and a CDRH3 comprising SEQ ID NO: 8 (ARLKGEFKDAFDI);
- (jj) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), a CDRH2 comprising SEQ ID NO: 26 (GRIIPLFGTAHYA), and a CDRH3 comprising SEQ ID NO: 8 (ARLKGEFKDAFDI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a
- 15 CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- (kk) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), a CDRH2 comprising SEQ ID NO: 27 (GRINPILGTANYA), and a CDRH3 comprising SEQ ID NO: 28 (ARLKGEFKDAFSI);
- 20 (ll) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), a CDRH2 comprising SEQ ID NO: 27 (GRINPILGTANYA), and a CDRH3 comprising SEQ ID NO: 28 (ARLKGEFKDAFSI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);
- 25 (mm) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), and a CDRH3 comprising SEQ ID NO: 30 (ARLKGEFKCAFDI);
- (nn) a CDRH1 comprising SEQ ID NO: 23 (GTFSSY AIS), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), and a comprising SEQ ID NO: 30
- 30 (ARLKGEFKCAFDI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT);

(oo) a CDRH1 comprising SEQ ID NO: 36 (GPFRSHAVS), a CDRH2 comprising SEQ ID NO: 11 (GGIIPVFGTATYA), and a CDRH3 comprising SEQ ID NO: 37 (ARLKSELKDAFDI); or

(pp) a CDRH1 comprising SEQ ID NO: 36 (GPFRSHAVS), a CDRH2 comprising
5 SEQ ID NO: 11 (GGIIPVFGTATYA), and a CDRH3 comprising SEQ ID NO: 37 (ARLKSELKDAFDI); a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT).

10 In some such examples, the second unit of antigen-binding binds PD-L1 and comprises and comprises a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to any one of SEQ ID NOs: 35, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, or 58, and a light chain variable region comprising an amino acid sequence that is at least 90% identical to any one of SEQ ID NOs: 59, 60, 61, 62, or 63.

15 In some examples, the second unit of antigen-binding binds PD-L1 and comprises a heavy chain variable region comprising:

- (a) an amino acid sequence that is at least 90% identical to SEQ ID NO: 35;
- (b) an amino acid sequence that is at least 90% identical to SEQ ID NO: 40;
- (c) an amino acid sequence that is at least 90% identical to SEQ ID NO: 41;
- 20 (d) an amino acid sequence that is at least 90% identical to SEQ ID NO: 42;
- (e) an amino acid sequence that is at least 90% identical to SEQ ID NO: 43;
- (f) an amino acid sequence that is at least 90% identical to SEQ ID NO: 44;
- (g) an amino acid sequence that is at least 90% identical to SEQ ID NO: 45;
- (h) an amino acid sequence that is at least 90% identical to SEQ ID NO: 46;
- 25 (i) an amino acid sequence that is at least 90% identical to SEQ ID NO: 47;
- (j) an amino acid sequence that is at least 90% identical to SEQ ID NO: 48;
- (k) an amino acid sequence that is at least 90% identical to SEQ ID NO: 49;
- (l) an amino acid sequence that is at least 90% identical to SEQ ID NO: 50;
- (m) an amino acid sequence that is at least 90% identical to SEQ ID NO: 51;
- 30 (n) an amino acid sequence that is at least 90% identical to SEQ ID NO: 52;
- (o) an amino acid sequence that is at least 90% identical to SEQ ID NO: 53;
- (p) an amino acid sequence that is at least 90% identical to SEQ ID NO: 54;
- (q) an amino acid sequence that is at least 90% identical to SEQ ID NO: 55;
- (r) an amino acid sequence that is at least 90% identical to SEQ ID NO: 56;

- (s) an amino acid sequence that is at least 90% identical to SEQ ID NO: 57; or
- (t) an amino acid sequence that is at least 90% identical to SEQ ID NO: 58;

In some examples, the second unit of antigen-binding binds PD-L1 and comprises a light chain variable region comprising:

- 5 (a) an amino acid sequence that is at least 90% identical to SEQ ID NO: 59;
- (b) an amino acid sequence that is at least 90% identical to SEQ ID NO: 60;
- (c) an amino acid sequence that is at least 90% identical to SEQ ID NO: 61;
- (d) an amino acid sequence that is at least 90% identical to SEQ ID NO: 62; or
- (e) an amino acid sequence that is at least 90% identical to SEQ ID NO: 63.

10 Also provided herein, in some examples and examples, is a multispecific antigen-binding construct comprising four units of antigen-binding, wherein two units of antigen-binding bind PD-1 and two units of antigen-binding bind PD-L1, and wherein the construct comprises a heavy chain amino acid sequence that is at least 85%, identical to the amino acid sequence of SEQ ID NO: 100 or 102, and a light chain amino acid sequence that is at least
15 85%, identical to the amino acid sequence of SEQ ID NO: 101 or 103.

Also provided herein, in some examples and examples, is a multispecific antigen-binding construct comprising four units of antigen-binding, wherein two units of antigen-binding bind PD-1 and two units of antigen-binding bind PD-L1, and wherein the construct comprises a heavy chain amino acid sequence that is at least 85%, identical to the amino acid
20 sequence of SEQ ID NO: 100 and a light chain amino acid sequence that is at least 85%, identical to the amino acid sequence of SEQ ID NO: 101.

Also provided herein, in some examples and examples, is a multispecific antigen-binding construct comprising four units of antigen-binding, wherein two units of antigen-binding bind PD-1 and two units of antigen-binding bind PD-L1, and wherein the construct
25 comprises a heavy chain amino acid sequence that is at least 85%, identical to the amino acid sequence of SEQ ID NO: 102 and a light chain amino acid sequence that is at least 85%, identical to the amino acid sequence of SEQ ID NO: 103.

In some examples, the construct does not comprise an Fc domain. In some examples, the first arm or second arm, or both, comprises a heavy chain comprising one or more
30 immunoglobulin Fc modifications. In some examples, the immunoglobulin Fc domain of the heavy chain comprises one or more amino acid mutations that promote heterodimerization of the first and second arms. In some examples, the mutation is present in a CH3 domain of the heavy chain. In some examples, the multispecific antigen-binding construct is produced in a quadroma cell. In some examples, the

construct comprises one or more immunoglobulin constant region modifications. In some examples, the immunoglobulin constant region comprises one or more amino acid mutations that promote heterodimerization of antibodies. In some examples, one or more mutations is present in the light chain constant region of one arm and one or more mutations is present in the heavy chain constant region of another arm. In some examples, the bispecific antibody is of a format selected from the group consisting of a bispecific IgG, bispecific antibody fragment, bispecific fusion protein, appended IgG, and bispecific antibody conjugate. In some examples, the Fc region has reduced effector function. In some examples, the Fc region enhances half-life of the construct.

In some examples, the construct comprises a heavy chain amino acid sequence that is at least 85%, identical to the amino acid sequence of SEQ ID NO: 100 or 102. In some examples, the construct comprises a light chain amino acid sequence that is at least 85%, identical to the amino acid sequence of SEQ ID NO: 101 or 103. In some examples, the construct comprises a heavy chain amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 100, and wherein the construct comprises a light chain amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 101. In some examples, the construct comprises a heavy chain amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 102, and wherein the construct comprises a light chain amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 103.

In some examples, any of the multispecific antigen-binding constructs disclosed herein are aglycosylated. In some examples, the multispecific antigen-binding construct is capable of binding human PD-1. In some examples, the multispecific antigen-binding construct is capable of binding murine PD-1. In some examples, the multispecific antigen-binding construct is capable of binding cynomolgus monkey PD-1. In some examples, the multispecific antigen-binding construct is capable of binding human, murine and cynomolgus monkey PD-1 with similar affinity.

In some examples, any of the multispecific antigen-binding constructs disclosed herein is capable of reducing PD-1 levels on a cell. In some examples, the multispecific antigen-binding construct is capable of inducing PD-1 degradation. In some examples, the multispecific antigen-binding construct is capable of reducing PD-1 expression. In some examples, the multispecific antigen-binding construct is capable of reducing PD-1 cell surface expression. In some examples, the multispecific antigen-binding construct is capable of reducing PD-1 cell surface expression by inducing shedding of PD-1 from the cell

surface. In some examples, the multispecific antigen-binding construct is capable of binding both PD-1 and PD-L1 and reducing PD-1 levels on a cell. In some examples, the multispecific antigen-binding construct is capable of binding both PD-1 and PD-L1 and inducing PD-1 degradation. In some examples, the multispecific antigen-binding construct is capable of binding both PD-1 and PD-L1 and reducing PD-1 expression. In some examples, the multispecific antigen-binding construct is capable of inducing shedding of PD-1 from an immune cell. In some examples, the multispecific antigen-binding construct is capable of binding both PD-1 and PD-L1 and inducing PD-1 shedding from an immune cell. In some examples, the multispecific antigen-binding construct is capable of sequestering PD-L1, such that PD-L1 cannot bind CD80. In some examples, the multispecific antigen-binding construct is capable of sequestering PD-L1, such that PD-L1 cannot bind CD80, and wherein CD80 is free to bind CD28. In some examples, the cell is an immune cell, such as a T cell. In some examples, the immune cell (*e.g.*, T cell) is a tumor infiltrating lymphocyte (TIL). In some examples, engagement of a multispecific antigen-binding molecule described herein to PD-1 expressed by an immune cell in the tumor microenvironment results in the downregulation of PD-1 by the immune cell. In some examples, the immune cell is a T cell. In some examples, the immune cell (*e.g.*, T cell) is a tumor infiltrating lymphocyte (TIL).

In some examples, any of the multispecific antigen-binding constructs disclosed herein is capable of inducing at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% greater interferon-gamma levels (*e.g.*, as measured in a *Staphylococcus aureus* Enterotoxin A (“SEA”) assay) as compared to a reference antigen-binding construct (*e.g.*, pembrolizumab or atezolizumab) or to a reference combination of antigen-binding constructs (*e.g.*, a composition comprising the PD-1 and PD-L1 arms of the multispecific antigen-binding construct, wherein the PD-1 and PD-L1 arms in the composition are not conjugated to one another). In some examples, the multispecific antigen-binding construct is capable of inducing at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% greater interleukin-2 levels (*e.g.*, as measured in a SEA assay) as compared to a reference antigen-binding construct (*e.g.*, pembrolizumab or atezolizumab) or to a reference combination of antigen-binding constructs (*e.g.*, a composition comprising the PD-1 and PD-L1 arms of the multispecific antigen-binding construct, wherein the PD-1 and PD-L1 arms in the composition are not conjugated to one another).

In some examples, the multispecific antigen-binding construct induces at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 200%, 300%, 400%, or 500% more killing of tumor cells (*e.g.*, leukemia cells, lymphoma cells, melanoma, or breast cancer cells), as compared to a reference antigen-binding construct (*e.g.*, pembrolizumab or atezolizumab) or to a reference combination of antigen-binding constructs (*e.g.*, a composition comprising the PD-1 and PD-L1 arms of the multispecific antigen-binding construct, wherein the PD-1 and PD-L1 arms in the composition are not conjugated to one another).

In some examples, the multispecific antigen-binding construct is capable of extending the survival of a subject suffering from a cancer (*e.g.*, leukemia, lymphoma, melanoma, and/or breast cancer) by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 100%, 200%, 300%, 400%, or 500% longer than a subject administered a reference antigen-binding construct (*e.g.*, pembrolizumab or atezolizumab) or a reference combination of antigen-binding constructs (*e.g.*, a composition comprising the PD-1 and PD-L1 arms of the multispecific antigen-binding construct, wherein the PD-1 and PD-L1 arms in the composition are not conjugated to one another). In some examples, the multispecific antigen-binding construct is capable of inducing at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 100%, 200%, 300%, 400%, or 500% more shedding of PD-1 from an immune cell as compared to an untreated immune cell or as compared to an immune cell treated with reference antigen-binding construct (*e.g.*, pembrolizumab or atezolizumab) or a reference combination of antigen-binding constructs (*e.g.*, a composition comprising the PD-1 and PD-L1 arms of the multispecific antigen-binding construct, wherein the PD-1 and PD-L1 arms in the composition are not conjugated to one another). In some examples, the multispecific antigen-binding construct is capable of reducing PD-1 levels at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 100%, 200%, 300%, 400%, or 500% (*e.g.*, by causing PD-1 shedding from the cell surface and/or inducing PD-1 degradation and/or reducing PD-1 expression) as compared to an untreated immune cell or to an immune cell treated with a reference antigen-binding construct (*e.g.*, pembrolizumab or atezolizumab) or a reference combination of antigen-binding constructs (*e.g.*, a composition comprising the PD-1 and PD-L1 arms of the multispecific antigen-binding construct, wherein the PD-1 and PD-L1 arms in the composition are not conjugated to one another).

In some examples, engagement of a multispecific antigen-binding molecule described herein to PD-1 expressed by a cell results in the downregulation and/or loss of cell-surface expression of PD-1 by the cell. Such down-regulation or loss of cell-surface expression can be due to, in part, for example, shedding of extracellular PD-1 from the surface of the immune cell. In some examples, the cell is an immune cell, such as a T cell. In some examples, the immune cell (e.g., T cell) is a tumor infiltrating lymphocyte (TIL). In some examples, engagement of a multispecific antigen-binding molecule described herein to PD-1 expressed by an immune cell in the tumor microenvironment results in the downregulation of PD-1 by the immune cell. In some examples, the immune cell is a T cell. In some examples, the immune cell (e.g., T cell) is a tumor infiltrating lymphocyte (TIL).

In some examples, the disclosure provides for a method for treating a proliferative disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of any of the multispecific antigen-binding constructs disclosed herein, thereby treating the proliferative disorder in the subject. In some examples, the proliferative disorder is cancer. In some examples, the cancer is selected from the group consisting of a hematological cancer, a neurological cancer, melanoma, breast cancer, lung cancer, head and neck cancer, a gastrointestinal cancer, liver cancer, pancreatic cancer, a genitourinary cancer, a bone cancer, and a vascular cancer. In some examples, the disclosure provides for a method of enhancing an immune response in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of any of the multispecific antigen-binding constructs disclosed herein, thereby enhancing the immune response in the subject. In some examples, the enhanced immune response includes any one or more of enhanced T cell function, enhanced NK cell function, or enhanced macrophage function. In some examples, the enhancement of T cell function is greater upon administration of the multispecific antigen-binding construct, as compared to an agent that binds either PD-1 or a PD-1 ligand, or a cocktail comprising an agent that binds PD-1 and an agent that binds a PD-1 ligand. In some examples, the T cell function is any one or more of increased IFN γ production from T cells, enhanced T cell survival, increased T cell proliferation, or rescue from an exhausted T cell phenotype. In some examples, the enhanced T cell function is greater upon administration of the multispecific antigen-binding construct, as compared to an agent that binds either PD-1 or a PD-1 ligand, or a cocktail comprising an agent that binds PD-1 and an agent that binds a PD-1 ligand. In some

examples, the multispecific antigen-binding construct is administered subcutaneously, intravenously, intradermally, intraperitoneally, orally, intramuscularly or intracranially. In some examples, the multispecific antigen-binding construct binds to PD-1 and to PD-L1 expressed on the surface of the same cell in the subject. In some examples, the multispecific antigen-binding construct binds to PD-1 expressed on the surface of a first cell in the subject, and wherein the multispecific antigen-binding construct binds to PD-L1 expressed on the surface of a second cell in the subject.

In some examples, the disclosure provides for an anti-PD1 antibody or antigen-binding fragment thereof, wherein the anti-PD-1 antibody or antigen-binding fragment comprises: (a) a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 70 (FTFX₁X₂YAX₃X₄, wherein X₁=S, R, G, or N; X₂=D, S, N, A, R, or G; X₃= M or L; X₄= S, L, or N); (ii) a CDRH2 comprising SEQ ID NO: 71 (SAISNSGTYTYYA); and (iii) a CDRH3 comprising SEQ ID NO: 72 (ARGLDFIVGX₅TGNDY, wherein X₅=A, Y, or R); and (b) a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT). In some examples, the anti-PD-1 antibody or antigen-binding fragment comprises: (a) a heavy chain variable region comprising (i) a CDRH1 comprising the amino acid sequence of any one of SEQ ID NOs: 73, 76, 77, 78, 79, 80, 81, 82, 83, 84, or 86; (ii) a CDRH2 comprising the amino acid sequence of SEQ ID NO: 71; and (iii) a CDRH3 comprising the amino acid sequence of any one of SEQ ID NOs: 74, 75, or 85; and (b) a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 9 (RASQSISSYLN); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 10 (QQSYSTPLT). In some examples, the heavy chain variable region comprises an amino acid sequence that is at least 85% identical to the amino acid sequence of any one of SEQ ID NOs: 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99. In some examples, the light chain variable region comprises an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 59.

In some examples, the disclosure provides for an anti-PD-L1 antibody or antigen-binding fragment thereof, wherein the anti-PD-L1 antibody or antigen-binding fragment comprises: a. a heavy chain variable region comprising (i) a CDRH1 comprising SEQ ID NO: 1 (GTFSSYAIN); (ii) a CDRH2 comprising SEQ ID NO: 2 (GGIIPX₁X₂GX₃ATYA, wherein X₁ is V or I; X₂ is F, L, or V; and X₃ is T or A); and (iii) a CDRH3 comprising SEQ ID NO: 3 (ARLKX₁ELKDAFDI, wherein X₁ is G, F, or N); and b. a light chain variable region comprising: (i) a CDRL1 comprising SEQ ID NO: 4 (RASQX₁ISSYLN, wherein X₁ is

S, W, or Q); (ii) a CDRL2 comprising SEQ ID NO: 5 (AASSLQS); and (iii) a CDRL3 comprising SEQ ID NO: 6 (X₁QSYSTPLT, wherein X₁ is Q or F). In some examples, the anti-PD-L1 antibody or antigen-binding fragment comprises: (a) a heavy chain variable region comprising (i) a CDRH1 comprising the amino acid sequence of any one of SEQ ID NOs: 1, 14, 23, 36, or 122; (ii) a CDRH2 comprising the amino acid sequence of any one of SEQ ID NOs: 11, 15, 16, 21, 24, 26, 27, 29, 31, 33, or 34; and (iii) a CDRH3 comprising the amino acid sequence of any one of SEQ ID NOs: 8, 17, 18, 19, 20, 22, 25, 28, 30, 32, or 37; and (b) a light chain variable region comprising: (i) a CDRL1 comprising the amino acid sequence of any one of SEQ ID NOs: 9, 12, or 13; (ii) a CDRL2 comprising the amino acid sequence of SEQ ID NO: 5; and (iii) a CDRL3 comprising the amino acid sequence of any one of SEQ ID NOs: 10, 38 or 39. In some examples, the heavy chain variable region comprises an amino acid sequence that is at least 85% identical to the amino acid sequence of any one of SEQ ID NOs: 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, or 35. In some examples, the light chain variable region comprises an amino acid sequence that is at least 85% identical to the amino acid sequence of any one of SEQ ID NOs: 59, 60, 61, 62, or 63.

In some examples, the disclosure provides for a method for treating a proliferative disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of any of the antibodies or antigen-binding constructs disclosed herein, thereby treating the proliferative disorder in the subject. In some examples, the proliferative disorder is cancer. In some examples, the cancer is selected from the group consisting of a hematological cancer, a neurological cancer, melanoma, breast cancer, lung cancer, head and neck cancer, a gastrointestinal cancer, liver cancer, pancreatic cancer, a genitourinary cancer, a bone cancer, and a vascular cancer.

In some examples, the disclosure provides for a method of enhancing an immune response in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of any of the antibodies or antigen-binding constructs disclosed herein, thereby enhancing the immune response in the subject.

In some examples, any of the methods described herein can involve detecting the presence or absence of PD-1 expression by one or more cells (or a population of cells, such as TILs) before and/or after contact with a multispecific antigen-binding molecule described herein. For example, any of the methods described herein can involve detecting the presence or absence of PD-1 expression by one or more cells (or a population of cells, such as TILs) before and/or after administration of a multispecific antigen-binding molecule described

herein to a subject (e.g., a cancer patient). Such methods are useful, e.g., determining a therapeutically effective amount of the molecule for use in treatment of a given patient or patient population. Methods of detecting the presence, reduction in expression, and/or absence of PD-1 expression are known to those of skill in the art, for example, using flow cytometry, Western blotting, ELISA, etc.

In another example, the disclosure features a method comprising measuring the level of PD-1 expression by one or more cells (or a population of cells, such as TILs) before and/or after contact with a multispecific antigen-binding molecule described herein. In some examples, the method comprises measuring the level of PD-1 expression by one or more cells (or a population of cells, such as TILs) before and/or after administration of a multispecific antigen-binding molecule described herein to a subject (e.g., a cancer patient).

In yet another example, the disclosure features a method comprising measuring the level of PD-1 expression by one or more cells (or a population of cells, such as TILs) before and/or after contact with a multispecific antigen-binding molecule described herein. For example, any of the methods described herein can involve measuring the level of PD-1 expression by one or more cells (or a population of cells, such as TILs) before and/or after administration of a multispecific antigen-binding molecule described herein to a subject (e.g., a cancer patient). Such methods are useful for, among other things, detecting or measuring a biological effect of a molecule described herein on the subject. In some examples, a reduction in the level of PD-1 expression by an immune cell (e.g., TILs isolated from a patient) following treatment with a multispecific antigen-binding molecule described herein indicates that the molecule has had a biological effect in the subject. In some examples, a reduction in the level of PD-1 expression by an immune cell (e.g., TILs isolated from a patient) following treatment with a multispecific antigen-binding molecule described herein indicates that the patient should receive one or more doses of the molecule, or otherwise continue on a therapy comprising the molecule.

In yet another example, the disclosure features methods for determining whether a biological effect has occurred in a patient or population of patients treated with a multispecific antigen-binding molecule described herein. The method comprises detecting the presence or amount of PD-1 expression by one or more test immune cells (e.g., effector immune cells, such as those in the tumor microenvironment) obtained from a patient or patients who have been administered a multispecific antigen-binding molecule described herein, wherein a reduced level of PD-1 expression (e.g., cell surface expression) of PD-1 by the one or more immune cells relative to a control expression level (e.g., the expression level

of PD-1 by immune cells of the same histological type as the test immune cells prior to administration of the molecule) indicates that a biological effect has occurred in the patient or population of patients. In some examples, the method comprises administering the multispecific antigen-binding molecule prior to the detecting. In some examples, the method
5 comprises administering a multispecific antigen-binding molecule to the patient or population of patients in whom the occurrence of a biological effect has been determined. In some examples, control PD-1 expression level is approximately the median or average expression level of PD-1 by immune cells of the same histological type in a population of subjects who have not been diagnosed as having a cancer. In some examples, control PD-1 expression
10 level is approximately the median or average expression level of PD-1 by immune cells of the same histological type in a population of subjects who have not been administered a multispecific antigen-binding molecule described herein and/or an agent that binds to and/or inhibits PD-1.

In yet another example, the disclosure features a method for reducing the expression
15 of PD-1 by one or more immune cells in a subject (*e.g.*, a cancer patient), the method comprising administering a multispecific antigen-binding molecule described herein to a subject to thereby reduce the expression of PD-1 by one or more immune cells in the subject. In some examples, the method comprises determining whether a reduction in PD-1 expression by one or more immune cells in the patient has occurred. In some examples, the
20 method comprises obtaining from the subject a biological sample (*e.g.*, a tumor biopsy) comprising one or more immune cells (*e.g.*, after administration of the molecule to the subject). In some examples, the method comprises measuring the level of PD-1 expression by one or more immune cells in the biological sample.

In yet another example, the disclosure features a method for inhibiting binding
25 between PD-L1 and CD80 in a subject (*e.g.*, a cancer patient), the method comprising administering a multispecific antigen-binding molecule described herein to a subject to thereby inhibit binding between PD-L1 and CD80 in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

30 The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1 shows the induction of interferon-gamma (IFN γ) in a mixed lymphocyte reaction (MLR) assay treated with various antibody cocktails or bispecific antibodies

WE CLAIM:

1. A multispecific antigen-binding construct comprising at least two antigen-binding arms, wherein:

a first arm binds PD-1 and comprises a heavy chain variable region and a light chain variable region, the heavy chain variable region of the first arm comprising a CDRH1 of SEQ ID NO: 77, a CDRH2 of SEQ ID NO: 71, and a CDRH3 of SEQ ID NO: 75, and

a second arm binds a PD-1 ligand and comprises a heavy chain variable region and a light chain variable region, the heavy chain variable region of the second arm comprising a CDRH1 of SEQ ID NO: 1, a CDRH2 of SEQ ID NO: 7, and a CDRH3 of SEQ ID NO: 8;

wherein the light chain variable region of the first arm and the light chain variable region of the second arm each comprise a CDRL1 of SEQ ID NO: 9, a CDRL2 of SEQ ID NO: 5, and a CDRL3 of SEQ ID NO: 10; and

wherein the multispecific antigen-binding construct blocks the interaction of PD-1 and the PD-1 ligand.

2. The multispecific antigen-binding construct of claim 1, wherein the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 90.

3. The multispecific antigen-binding construct of claim 1, wherein the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 90.

4. The multispecific antigen-binding construct of claim 1, wherein the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 55.

5. The multispecific antigen-binding construct of claim 1, wherein the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 55.
6. The multispecific antigen-binding construct of claim 1, wherein the first and the second antigen-binding arms each comprise a common light chain variable region.
7. The multispecific antigen-binding construct of claim 1, wherein the construct comprises at least two arms that bind PD-1 and at least two arms that bind PD-L1.
8. The multispecific antigen-binding construct of claim 1, wherein the construct comprises a bivalent antibody specific for PD-1, and a bivalent antibody specific for PD-L1, wherein the bivalent antibody specific for PD-1 and the bivalent antibody specific for PD-L1 are linked.
9. The multispecific antigen-binding construct of claim 1, wherein the heavy chain of the first arm has an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 100; and wherein the light chain of the first arm and the second arm has an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 101.
10. The multispecific antigen-binding construct of claim 1, wherein heavy chain of the first arm has an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 100, and wherein the light chain of the first arm and the second arm have an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 101.
11. The multispecific antigen-binding construct of claim 1, wherein the heavy chain of the first arm has an amino acid sequence of SEQ ID NO: 100, and wherein the light chain of

the first arm and the second arm have an amino acid sequence of SEQ ID NO: 101.

12. The multispecific antigen-binding construct of claim 1, wherein the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 90.

13. The multispecific antigen-binding construct of claim 1, wherein the heavy chain variable region of the first arm comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 90.

14. The multispecific antigen-binding construct of claim 1, wherein the heavy chain variable region of the first arm comprises the amino acid sequence of SEQ ID NO: 90.

15. The multispecific antigen-binding construct of claim 1, wherein the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 55.

16. The multispecific antigen-binding construct of claim 1, wherein the heavy chain variable region of the second arm comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 55.

17. The multispecific antigen-binding construct of claim 1, wherein the heavy chain variable region of the second arm comprises the amino acid sequence of SEQ ID NO: 55.

18. The multispecific antigen-binding construct of claim 1, wherein the heavy chain of the first arm has an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 100, and wherein the light chain of the first arm and the second arm have an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 101.

19. The multispecific antigen-binding construct of claim 1, wherein the heavy chain of the first arm has an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 100, and wherein the light chain of the first arm and the second arm have an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 101.

20. The multispecific antigen-binding construct of claim 1, wherein the first arm binds PD-1 expressed by an immune cell.

21. The multispecific antigen-binding construct of claim 1, wherein the second arm binds a PD-1 ligand expressed by a second cell.

22. The multispecific antigen-binding construct of claim 21, wherein the second cell is a tumor cell and the PD-1 ligand is PD-L1.