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Canadian Intellectual Property Office

CA 3091801 A1 2019/09/06

(21) 3 091 801

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2019/03/01
(87) Date publication PCT/PCT Publication Date: 2019/09/06
(85) Entrée phase nationale/National Entry: 2020/08/19
(86) N° demande PCT/PCT Application No.: US 2019/020189
(87) N° publication PCT/PCT Publication No.: 2019/169212
(30) Priorité/Priority: 2018/03/02 (US62/637,740)

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(54) Titre : ANTICORPS B7-H4 ET LEURS PROCEDES D'UTILISATION
(54) Title: B7-H4 ANTIBODIES AND METHODS OF USE THEREOF

(57) Abrégé/Abstract:
The present disclosure provides antibodies and antigen-binding fragments thereof that specifically bind to human B7-H4. The anti-B7-H4 antibodies or antigen-binding fragments thereof are useful, for example in detecting B7-H4. Immunohistochemistry (IHC) can be used to detect B7-H4. The present disclosure also provides methods for treating cancer wherein increased B7-H4 has been detected, by administering a therapeutic anti-B7-H4 antibody or antigen-binding fragment thereof.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
06 September 2019 (06.09.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/169212 A1

(51) International Patent Classification:
C07K 16/28 (2006.01) *A61P 37/00* (2006.01)

(21) International Application Number:
PCT/US2019/020189

(22) International Filing Date:
01 March 2019 (01.03.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/637,740 02 March 2018 (02.03.2018) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2019/169212 A1

(54) Title: B7-H4 ANTIBODIES AND METHODS OF USE THEREOF

(57) Abstract: The present disclosure provides antibodies and antigen-binding fragments thereof that specifically bind to human B7-H4. The anti-B7-H4 antibodies or antigen-binding fragments thereof are useful, for example in detecting B7-H4. Immunohistochemistry (IHC) can be used to detect B7-H4. The present disclosure also provides methods for treating cancer wherein increased B7-H4 has been detected, by administering a therapeutic anti-B7-H4 antibody or antigen-binding fragment thereof.

B7-H4 ANTIBODIES AND METHODS OF USE THEREOF

FIELD

[0001] The present disclosure relates to antibodies that specifically bind to human B7-H4 and methods of producing and using such antibodies, for example to detect B7-H4.

BACKGROUND

[0002] B7-H4 (also known as B7x, B7-S1, and VTCN1) is an immune regulatory molecule that shares homology with other B7 family members, include PD-L1. It is a type I transmembrane protein comprised of both IgV and IgC ectodomains. While B7-H4 expression in healthy tissues is relatively limited at the protein level, B7-H4 is expressed in several solid tumors such as gynecological carcinomas of the breast, ovary, and endometrium. Expression of B7-H4 in tumors tends to correlate with poor prognosis. The receptor for B7-H4 is unknown, but it is believed to be expressed on T cells. B7-H4 is believed to directly inhibit T cell activity.

[0003] Given the expression and function of B7-H4, antibodies that specifically bind to B7-H4 and the use of these antibodies to detect B7-H4, including, *e.g.*, using immunohistochemistry (IHC) to detect B7-H4 in cancer samples, are needed.

SUMMARY

[0004] Provided herein are antibodies that specifically bind to B7-H4 and the use of these antibodies to detect B7-H4, including, e.g., using immunohistochemistry (IHC) to detect B7-H4 in cancer samples.

[0005] In certain embodiments, provided herein is an isolated antibody or antigen-binding fragment thereof that specifically binds to human B7-H4, comprising a heavy chain variable region (VH) complementarity determining region (CDR) 1 comprising the amino acid sequence of SEQ ID NO:22, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:23, a VH CDR3 comprising the amino acid sequence of SEQ ID NO:24, a light chain variable region (VL) CDR1 comprising the amino acid sequence of SEQ ID NO:25, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:26, 27, or 28, and a VL CDR3 sequence comprising the amino acid sequence of SEQ ID NO:29.

[0006] In certain embodiments, the antibody or antigen-binding fragment thereof comprises a VH comprising the amino acid sequence of SEQ ID NO:6, 7, or 8. In certain embodiments, the antibody or antigen-binding fragment thereof comprises a VL comprising the amino acid sequence of SEQ ID NO:9, 10, or 11.

[0007] In certain embodiments, provided herein is an isolated antibody or antigen-binding fragment thereof that specifically binds to human B7-H4, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:6, 7, or 8.

[0008] In certain embodiments, provided herein is an isolated antibody or antigen-binding fragment thereof that specifically binds to human B7-H4, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO:9, 10, or 11.

[0009] In certain embodiments, provided herein is an isolated antibody or antigen-binding fragment thereof that specifically binds to human B7-H4, comprising a heavy chain variable region and a light chain variable region comprising the amino acid sequences of: (a) SEQ ID NOs:6 and 9, respectively; (b) SEQ ID NOs:6 and 10, respectively; (c) SEQ ID NOs:6 and 11, respectively; (d) SEQ ID NOs:7 and 9, respectively; (e) SEQ ID NOs:7 and 10, respectively; (f) SEQ ID NOs:7 and 11, respectively; (g) SEQ ID NOs:8 and 9, respectively; (h) SEQ ID NOs:8 and 10, respectively; (i) SEQ ID NOs:8 and 11, respectively, (j) SEQ ID NOs: 89 and 19, respectively; (k) SEQ ID NOs: 90 and 19, respectively; or (l) SEQ ID NOs: 91 and 19, respectively.

[0010] In certain embodiments, the antibody or antigen-binding fragment thereof further comprises a heavy chain constant region. In certain embodiments, the heavy chain constant region is a murine IgG_1 or IgG_{2a} heavy chain constant region.

[0011] In certain embodiments, the antibody or antigen-binding fragment further comprises a light chain constant region. In certain embodiments, light chain constant region is a murine IgGκ light chain constant region.

[0012] In certain embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:16, 17, 18,

89, 90, or 91. In certain embodiments, the antibody or antigen-binding fragment thereof comprises a light chain comprising the amino acid sequence of SEQ ID NO:19, 30, or 31.

[0013] In certain embodiments, the antibody or antigen-binding fragment comprises a heavy chain and a light chain comprising the amino acid sequences of: (a) SEQ ID NOs:16 and 19, respectively; (b) SEQ ID NOs:16 and 30, respectively; (c) SEQ ID NOs:16 and 31, respectively; (d) SEQ ID NOs:17 and 19, respectively; (e) SEQ ID NOs:17 and 30, respectively; (f) SEQ ID NOs:17 and 31, respectively; (g) SEQ ID NOs:18 and 19, respectively; (h) SEQ ID NOs:18 and 30, respectively; or (i) SEQ ID NOs:18 and 31, respectively.

[0014] In certain embodiments, provided herein is an isolated antibody or antigen-binding fragment thereof that specifically binds to human B7-H4, wherein the antibody or antigen-binding fragment thereof comprises the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 of an antibody selected from the group consisting of J512, J513, J514, J515, J516, J517, J518, J519, J520, J521, and J522. In certain embodiments, the CDRs are the Kabat-defined CDRs, the Chothia-defined CDRs, or the AbM-defined CDRs.

[0015] In certain embodiments, provided herein is an isolated antibody or antigen-binding fragment thereof that specifically binds to the same epitope of human B7-H4 as an antibody or antigen-binding fragment thereof provided herein.

[0016] In certain embodiments, provided herein is an isolated antibody or antigen-binding fragment thereof that competitively inhibits binding of the antibody or antigen-binding fragment thereof of another antibody or antigen-binding fragment thereof provided herein to human B7-H4.

[0017] In certain embodiments, the antibody or antigen-binding fragment thereof is a murine antibody or antigen-binding fragment thereof.

[0018] In certain embodiments, the antibody or antigen-binding fragment thereof is a full length antibody. In certain embodiments, the antibody or antigen-binding fragment thereof is an antigen binding fragment. In certain embodiments, the antigen binding fragment comprises a Fab, Fab', $F(ab')_2$, single chain Fv (scFv), disulfide linked Fv, minibody, $F(ab')_3$, diabody, $(scFv)_2$, or scFv-Fc.

[0019] In certain embodiments, the antibody or antigen-binding fragment thereof further comprises a detectable label.

[0020] In certain embodiments, provided herein is an isolated polynucleotide comprising a nucleic acid molecule encoding the heavy chain variable region or heavy chain of an antibody or antigen-biding fragment thereof provided herein. In certain embodiments, the nucleic acid molecule encodes the VH of SEQ ID NO:6, 7, or 8 or the heavy chain of SEQ ID NO:16, 17, or 18.

[0021] In certain embodiments, the nucleic acid molecule comprises the sequence of SEQ ID NO:12, 13, 14, 93, 94, or 95.

[0022] In certain embodiments, provided herein is an isolated polynucleotide comprising a nucleic acid molecule encoding the light chain variable region or light chain of an antibody or antigen-biding fragment thereof provided herein. In certain embodiments, the nucleic acid molecule encodes the VL of SEQ ID NO:9, 10, or 11 or the light chain of SEQ ID NO:19, 30, or 31. In certain embodiments, the nucleic acid molecule comprises the sequence of SEQ ID NO:15, 96, or 97.

[0023] In certain embodiments, provided herein is an isolated polynucleotide comprising a nucleic acid molecule encoding the heavy chain variable region or heavy chain of an antibody or antigen-binding fragment thereof provided herein and the light chain variable region or light chain of the antibody or antigen-binding fragment thereof provided herein.

[0024] In certain embodiments, provided herein is an isolated vector comprising a polynucleotide provided herein.

[0025] In certain embodiments, provided herein is a host cell (e.g., an isolated host cell) comprising a polynucleotide provided herein, a vector provided herein, or a first vector comprising a polynucleotide encoding a light chain variable region or light chain provided herein and a second vector comprising a polynucleotide encoding a heavy chain variable region or heavy chain provided herein. In certain embodiments, the host cell is a CHO or HEK cell.

[0026] In certain embodiments, provided herein is a method of producing an antibody or antigen-binding fragment thereof that specifically binds to human B7-H4 comprising culturing a host cell of provided herein so that the nucleic acid molecule is expressed and the antibody or antigen-biding fragment thereof is produced.

[0027] In certain embodiments, provided herein is an isolated antibody or antigen-binding fragment thereof that specifically binds to human B7-H4 and is encoded by a polynucleotide provided herein.

[0028] In certain embodiments, provided herein is a method for detecting B7-H4 in a sample comprising contacting the sample with an antibody or antigen-binding fragment thereof provided herein. In certain embodiments, provided herein is a method for detecting B7-H4 in a sample comprising contacting the sample with an anti-B7-H4 antibody or antigen-binding fragment thereof provided herein and detecting binding of the antibody or antigen-binding fragment thereof to B7-H4. In certain embodiments, the sample is obtained from a cancer in a subject. In certain embodiments, the method further comprises administering a therapeutic anti-B7-H4 antibody or antigen-binding fragment thereof to the subject after B7-H4 has been detected.

[0029] In certain embodiments, provided herein is a method of treating a B7-H4 expressing cancer in a subject, the method comprising administering to the subject a therapeutic anti-B7-H4 antibody or antigen-binding fragment thereof, wherein B7-H4 was detected in a sample obtained from the cancer using an antibody or antigen-binding fragment thereof provided herein.

[0030] In certain embodiments, the method further comprises detecting the B7-H4 in the sample obtained from the cancer.

[0031] In certain embodiments, the detected B7-H4 is cell membrane B7-H4. In certain embodiments, the detected B7-H4 is cytoplasmic B7-H4. In certain embodiments, the detected B7-H4 is whole-cell B7-H4.

[0032] In certain embodiments, the B7-H4 is detected in circulating tumor cells.

[0033] In certain embodiments, the sample is solid tissue, biopsy, ascites, an aspirate, a fluidic extract, blood plasma, serum, spinal fluid, lymph fluid, the external section of the skin, respiratory, intestinal, or genitourinary tract, tears, saliva, milk, a tumor, or an organ from a subject.

[0034] In certain embodiments, the cancer is selected from the group consisting of breast cancer, ductal carcinoma, endometrial carcinoma, ovarian cancer, non-small cell lung cancer, pancreatic cancer, thyroid cancer, kidney cancer and bladder cancer. In certain embodiments, the breast cancer is triple negative breast cancer or wherein the non-small cell lung cancer is squamous cell carcinoma.

[0035] In certain embodiments, the method of detection uses an enzyme linked immunosorbent assay (ELISA), a fluorescence-activated cell sorter (FACS) assay, or immunohistochemistry (IHC). In certain embodiments, the method of detection uses IHC

and the concentration of the antibody or antigen-binding fragment thereof provided herein is about 1 to about 50 μg/ml. In certain embodiments, the concentration of the antibody or antigen-binding fragment thereof provided herein is about 1 to about 20 μg/ml. In certain embodiments, the concentration of the antibody or antigen-binding fragment thereof provided herein is about 10 μg/mL.

[0036] In certain embodiments, the subject is human.

[0037] In certain embodiments, provided herein is a kit comprising an antibody or antigen-binding fragment thereof provided herein and a) a detection reagent, b) a B7-H4 antigen, c) a therapeutic anti-B7-H4 antibody, or d) a combination thereof.

[0038] In certain embodiments of the method or kit provided herein, the therapeutic antibody or antigen-binding fragment comprises the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 of 20502 or 22213. In certain embodiments, the CDRs are the Kabat-defined CDRs, the Chothia-defined CDRs, or the AbM-defined CDR.

[0039] In certain embodiments, the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 comprise the amino acid sequences of SEQ ID NOs:32-37, respectively or the amino acid sequences of SEQ ID NOs:58-63, respectively. In certain embodiments, the therapeutic antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:54 or SEQ ID NO:64. In certain embodiments, the therapeutic antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO:55 or SEQ ID NO:65. In certain embodiments, the therapeutic antibody or antigen-binding fragment comprises (i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:54 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:55 or (ii) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:64 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:65. In certain embodiments, the therapeutic antibody or antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:56 or SEQ ID NO:74. In certain embodiments, the therapeutic antibody or antigen-binding fragment thereof comprises a light chain comprising the amino acid sequence of SEQ ID

NO:57 or SEQ ID NO:75. In certain embodiments, the therapeutic antibody or antigen-binding fragment thereof comprises (i) a heavy chain comprising the amino acid sequence of SEQ ID NO:56 and a light chain comprising the amino acid sequence of SEQ ID NO:57 or (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO:74 and a light chain comprising the amino acid sequence of SEQ ID NO:75.

BRIEF DESCRIPTION OF THE FIGURES

[0040] **Figure 1** shows IHC staining images generated using the anti-B7-H4 antibodies A57.1, AET_AB_J516, and AET_AB_J512. (See Example 4.)

[0041] **Figure 2** shows computational image analysis (Definiens) of IHC staining. (See Example 4.)

[0042] **Figure 3** shows the segmentation of positive cells with different intensity levels. (See Example 4.)

[0043] **Figure 4** shows the number and proportion of cells associated with each staining intensity level obtained using the anti-B7-H4 antibodies A57.1, AET_AB_J516, and AET_AB_J512. (See Example 4.)

[0044] **Figures 5A-5C** show a comparison of B7-H4 expression (DAB intensity) in whole cells, membranes, and cytoplasms of B7-H4 positive cells. Figure 5A shows the B7-H4 expression (DAB intensity) using A57.1 antibody. Figure 5B shows the B7-H4 expression (DAB intensity) using J512 antibody. Figure 5C shows the B7-H4 expression (DAB intensity) using J516 antibody. (See Example 4.)

DETAILED DESCRIPTION

[0045] Provided herein are antibodies (*e.g.*, monoclonal antibodies) and antigen-binding fragments thereof that specifically bind to B7-H4 (*e.g.*, human B7-H4). The anti-B7-H4 antibodies and antigen-binding fragments thereof can be used to detect B7-H4, for example, using immunohistochemistry on a cancer sample.

[0046] Also provided are isolated nucleic acids (polynucleotides), such as complementary DNA (cDNA), encoding such antibodies and antigen-binding fragments thereof. Further provided are vectors (*e.g.*, expression vectors) and cells (*e.g.*, host cells) comprising nucleic acids (polynucleotides) encoding such antibodies and antigen-binding fragments

thereof. Also provided are methods of making such antibodies and antigen-binding fragments thereof. In other aspects, provided herein are methods for detecting B7-H4, *e.g.*, in a cancer sample. Related compositions (*e.g.*, detection compositions), and kits are also provided.

Terminology

[0047] As used herein, the term "B7-H4" refers to mammalian B7-H4 polypeptides including, but not limited to, native B7-H4 polypeptides and isoforms of B7-H4 polypeptides. "B7-H4" encompasses full-length, unprocessed B7-H4 polypeptides as well as forms of B7-H4 polypeptides that result from processing within the cell. As used herein, the term "human B7-H4" refers to a polypeptide comprising the amino acid sequence of SEQ ID NO:1. A "B7-H4 polynucleotide," "B7-H4 nucleotide," or "B7-H4 nucleic acid" refer to a polynucleotide encoding B7-H4.

[0048] The term "antibody" means an immunoglobulin molecule that recognizes and specifically binds to a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing through at least one antigen recognition site within the variable region of the immunoglobulin molecule. As used herein, the term "antibody" encompasses intact polyclonal antibodies, intact monoclonal antibodies, chimeric antibodies, humanized antibodies, human antibodies, fusion proteins comprising an antibody, and any other modified immunoglobulin molecule so long as the antibodies exhibit the desired biological activity. An antibody can be of any the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (*e.g.* IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their heavy-chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. The different classes of immunoglobulins have different and well known subunit structures and three-dimensional configurations. Antibodies can be naked or conjugated to other molecules such as toxins, radioisotopes, etc.

[0049] The term "antibody fragment" refers to a portion of an intact antibody. An "antigen-binding fragment," "antigen-binding domain," or "antigen-binding region," refers to a portion of an intact antibody that specifically binds to an antigen. An antigen-binding fragment can contain an antigen recognition site of an intact antibody (*e.g.*, complementarity determining regions (CDRs) sufficient to specifically bind antigen). Examples of antigen-binding fragments of antibodies include, but are not limited to Fab,

Fab', F(ab')2, and Fv fragments, linear antibodies, and single chain antibodies. An antigen-binding fragment of an antibody can be derived from any animal species, such as rodents (*e.g.*, mouse, rat, or hamster) and humans or can be artificially produced.

[0050] The terms "anti-B7-H4 antibody," "B7-H4 antibody" and "antibody that binds to B7-H4" refer to an antibody that is capable of specifically binding B7-H4 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting B7-H4. As used herein, the terms “specifically binding,” “immunospecifically binding,” “immunospecifically recognizing,” and “specifically recognizing” are analogous terms in the context of antibodies or antigen-binding fragments thereof. These terms indicate that the antibody or antigen-binding fragment thereof binds to an epitope via its antigen-binding domain and that the binding entails some complementarity between the antigen binding domain and the epitope. Accordingly, an antibody that “specifically binds” to human B7-H4 (SEQ ID NO:1) may also bind to B7-H4 from other species (e.g., cynomolgus monkey, mouse, and/or rat B7-H4) and/or B7-H4 proteins produced from other human alleles, but the extent of binding of an anti-B7-H4 antibody to an unrelated, non-B7-H4 protein (e.g., other B7 protein family members such as PD-L1) is less than about 10% of the binding of the antibody to B7-H4 as measured, *e.g.*, by a radioimmunoassay (RIA).

[0051] A "monoclonal" antibody or antigen-binding fragment thereof refers to a homogeneous antibody or antigen-binding fragment population involved in the highly specific recognition and binding of a single antigenic determinant, or epitope. This is in contrast to polyclonal antibodies that typically include different antibodies directed against different antigenic determinants. The term "monoclonal" antibody or antigen-binding fragment thereof encompasses both intact and full-length monoclonal antibodies as well as antibody fragments (such as Fab, Fab', F(ab')2, Fv), single chain (scFv) mutants, fusion proteins comprising an antibody portion, and any other modified immunoglobulin molecule comprising an antigen recognition site. Furthermore, “monoclonal" antibody or antigen-binding fragment thereof refers to such antibodies and antigen-binding fragments thereof made in any number of manners including but not limited to by hybridoma, phage selection, recombinant expression, and transgenic animals.

[0052] As used herein, the terms "variable region" or "variable domain" are used interchangeably and are common in the art. The variable region typically refers to a portion of an antibody, generally, a portion of a light or heavy chain, typically about the amino-terminal 110 to 120 amino acids or 110 to 125 amino acids in the mature heavy chain and about 90 to 115 amino acids in the mature light chain, which differ in sequence among antibodies and are used in the binding and specificity of a particular antibody for its particular antigen. The variability in sequence is concentrated in those regions called complementarity determining regions (CDRs) while the more highly conserved regions in the variable domain are called framework regions (FR). Without wishing to be bound by any particular mechanism or theory, it is believed that the CDRs of the light and heavy chains are primarily responsible for the interaction and specificity of the antibody with antigen. In certain embodiments, the variable region is a human variable region. In certain embodiments, the variable region is a rodent or murine variable region.

[0053] The terms "VL" and "VL domain" are used interchangeably to refer to the light chain variable region of an antibody.

[0054] The terms "VH" and "VH domain" are used interchangeably to refer to the heavy chain variable region of an antibody.

[0055] The term "Kabat numbering" and like terms are recognized in the art and refer to a system of numbering amino acid residues in the heavy and light chain variable regions of an antibody or an antigen-binding fragment thereof. In certain aspects, CDRs can be determined according to the Kabat numbering system (see, *e.g.*, Kabat EA & Wu TT (1971) Ann NY Acad Sci 190: 382-391 and Kabat EA *et al.*, (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Using the Kabat numbering system, CDRs within an antibody heavy chain molecule are typically present at amino acid positions 31 to 35, which optionally can include one or two additional amino acids, following 35 (referred to in the Kabat numbering scheme as 35A and 35B) (CDR1), amino acid positions 50 to 65 (CDR2), and amino acid positions 95 to 102 (CDR3). Using the Kabat numbering system, CDRs within an antibody light chain molecule are typically present at amino acid positions 24 to 34 (CDR1), amino acid positions 50 to 56 (CDR2), and amino acid positions 89 to 97 (CDR3). In a specific embodiment, the CDRs of the antibodies described herein have been determined according to the Kabat numbering scheme.

[0056] Chothia refers instead to the location of the structural loops (Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987)). The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software.

Loop	Kabat	AbM	Chothia
L1	L24-L34	L24-L34	L24-L34
L2	L50-L56	L50-L56	L50-L56
L3	L89-L97	L89-L97	L89-L97
H1	H31-H35B	H26-H35B (Kabat Numbering)	H26-H32..34
H1	H31-H35	H26-H35 (Chothia Numbering)	H26-H32
H2	H50-H65	H50-H58	H52-H56
H3	H95-H102	H95-H102	H95-H102

[0057] As used herein, the terms "constant region" and "constant domain" are interchangeable and have their common meanings the art. The constant region is an antibody portion, *e.g.*, a carboxyl terminal portion of a light and/or heavy chain which is not directly involved in binding of an antibody to antigen but which can exhibit various effector functions, such as interaction with the Fc receptor. The constant region of an immunoglobulin molecule generally has a more conserved amino acid sequence relative to an immunoglobulin variable domain. In certain aspects, an antibody or antigen-binding fragment comprises a constant region or portion thereof that is sufficient for antibody-dependent cell-mediated cytotoxicity (ADCC).

[0058] As used herein, the term "heavy chain" when used in reference to an antibody can refer to any distinct type, *e.g.*, alpha (α), delta (δ), epsilon (ε), gamma (γ), and mu (μ), based on the amino acid sequence of the constant domain, which give rise to IgA, IgD, IgE, IgG, and IgM classes of antibodies, respectively, including subclasses of IgG, *e.g.*, IgG_1, IgG_2, IgG_3, and IgG_4. Heavy chain amino acid sequences are well known in the art.

In specific embodiments, the heavy chain is a human heavy chain. In specific embodiments, the heavy chain is a rodent or murine heavy chain.

[0059] As used herein, the term "light chain" when used in reference to an antibody can refer to any distinct type, *e.g.*, kappa (κ) or lambda (λ) based on the amino acid sequence of the constant domains. Light chain amino acid sequences are well known in the art. In specific embodiments, the light chain is a human light chain. In specific embodiments, the light chain is a rodent or murine light chain.

[0060] The term "chimeric" antibodies or antigen-binding fragments thereof refers to antibodies or antigen-binding fragments thereof wherein the amino acid sequence is derived from two or more species. Typically, the variable region of both light and heavy chains corresponds to the variable region of antibodies or antigen-binding fragments thereof derived from one species of mammals (*e.g.* mouse, rat, rabbit, etc.) with the desired specificity, affinity, and capability while the constant regions are homologous to the sequences in antibodies or antigen-binding fragments thereof derived from another (usually human) to avoid eliciting an immune response in that species.

[0061] The term "humanized" antibody or antigen-binding fragment thereof refers to forms of non-human (*e.g.* murine) antibodies or antigen-binding fragments that are specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human (*e.g.*, murine) sequences. Typically, humanized antibodies or antigen-binding fragments thereof are human immunoglobulins in which residues from the complementary determining region (CDR) are replaced by residues from the CDR of a non-human species (*e.g.* mouse, rat, rabbit, hamster) that have the desired specificity, affinity, and capability ("CDR grafted") (Jones *et al.*, Nature 321:522-525 (1986); Riechmann *et al.*, Nature 332:323-327 (1988); Verhoeyen *et al.*, Science 239:1534-1536 (1988)). In some instances, certain Fv framework region (FR) residues of a human immunoglobulin are replaced with the corresponding residues in an antibody or fragment from a non-human species that has the desired specificity, affinity, and capability. The humanized antibody or antigen-binding fragment thereof can be further modified by the substitution of additional residues either in the Fv framework region and/or within the non-human CDR residues to refine and optimize antibody or antigen-binding fragment thereof specificity, affinity, and/or capability. In general, the humanized antibody or antigen-binding fragment thereof will comprise variable domains containing all or

substantially all of the CDR regions that correspond to the non-human immunoglobulin whereas all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody or antigen-binding fragment thereof can also comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Examples of methods used to generate humanized antibodies are described in U.S. Pat. 5,225,539; Roguska *et al.*, Proc. Natl. Acad. Sci., USA, 91(3):969-973 (1994), and Roguska *et al.*, Protein Eng. 9(10):895-904 (1996). In some embodiments, a "humanized antibody" is a resurfaced antibody.

[0062] The term "human" antibody or antigen-binding fragment thereof means an antibody or antigen-binding fragment thereof having an amino acid sequence derived from a human immunoglobulin gene locus, where such antibody or antigen-binding fragment is made using any technique known in the art. This definition of a human antibody or antigen-binding fragment thereof includes intact or full-length antibodies and fragments thereof.

[0063] "Binding affinity" generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (*e.g.*, an antibody or antigen-binding fragment thereof) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody or antigen-binding fragment thereof and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured and/or expressed in a number of ways known in the art, including, but not limited to, equilibrium dissociation constant (K_D), and equilibrium association constant (K_A). The K_D is calculated from the quotient of k_{off}/k_{on}, whereas K_A is calculated from the quotient of k_{on}/k_{off}. k_{on} refers to the association rate constant of, *e.g.*, an antibody or antigen-binding fragment thereof to an antigen, and k_{off} refers to the dissociation of, *e.g.*, an antibody or antigen-binding fragment thereof from an antigen. The k_{on} and k_{off} can be determined by techniques known to one of ordinary skill in the art, such as BIAcore® or KinExA.

[0064] As used herein, an "epitope" is a term in the art and refers to a localized region of an antigen to which an antibody or antigen-binding fragment thereof can specifically bind. An epitope can be, for example, contiguous amino acids of a polypeptide (linear or

contiguous epitope) or an epitope can, for example, come together from two or more non-contiguous regions of a polypeptide or polypeptides (conformational, non-linear, discontinuous, or non-contiguous epitope). In certain embodiments, the epitope to which an antibody or antigen-binding fragment thereof specifically binds can be determined by, *e.g.*, NMR spectroscopy, X-ray diffraction crystallography studies, ELISA assays, hydrogen/deuterium exchange coupled with mass spectrometry (*e.g.*, liquid chromatography electrospray mass spectrometry), array-based oligo-peptide scanning assays, and/or mutagenesis mapping (*e.g.*, site-directed mutagenesis mapping). For X-ray crystallography, crystallization may be accomplished using any of the known methods in the art (*e.g.*, Giegé R *et al.,* (1994) Acta Crystallogr D Biol Crystallogr 50(Pt 4): 339-350; McPherson A (1990) Eur J Biochem 189: 1-23; Chayen NE (1997) Structure 5: 1269-1274; McPherson A (1976) J Biol Chem 251: 6300-6303). Antibody/antigen-binding fragment thereof: antigen crystals can be studied using well known X-ray diffraction techniques and can be refined using computer software such as X-PLOR (Yale University, 1992, distributed by Molecular Simulations, Inc.; *see*, *e.g.*, Meth Enzymol (1985) volumes 114 & 115, eds Wyckoff HW *et al.*,; U.S. 2004/0014194), and BUSTER (Bricogne G (1993) Acta Crystallogr D Biol Crystallogr 49(Pt 1): 37-60; Bricogne G (1997) Meth Enzymol 276A: 361-423, ed Carter CW; Roversi P *et al.,* (2000) Acta Crystallogr D Biol Crystallogr 56(Pt 10): 1316-1323). Mutagenesis mapping studies can be accomplished using any method known to one of skill in the art. *See*, *e.g.*, Champe M *et al.,* (1995) J Biol Chem 270: 1388-1394 and Cunningham BC & Wells JA (1989) Science 244: 1081-1085 for a description of mutagenesis techniques, including alanine scanning mutagenesis techniques.

[0065] A B7-H4 antibody that "binds to the same epitope" as a reference B7-H4 antibody refers to an antibody that binds to the same B7-H4 amino acid residues as the reference B7-H4 antibody. The ability of a B7-H4 antibody to bind to the same epitope as a reference B7-4 antibody is determined by a hydrogen/deuterium exchange assay (see Coales *et al*. Rapid Commun. Mass Spectrom. 2009; 23: 639–647).

[0066] An antibody is said to "competitively inhibit" binding of a reference antibody to a given epitope if it binds to the same epitope or an overlapping epitope of the reference antibody such that it blocks, to some degree, binding of the reference antibody to the epitope. Competitive inhibition may be determined by any method known in the art, for

example, competition ELISA assays. An antibody may be said to competitively inhibit binding of the reference antibody to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

[0067] A polypeptide, antibody, polynucleotide, vector, cell, or composition which is "isolated" is a polypeptide, antibody, polynucleotide, vector, cell, or composition which is in a form not found in nature. Isolated polypeptides, antibodies, polynucleotides, vectors, cell or compositions include those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some embodiments, an antibody, polynucleotide, vector, cell, or composition which is isolated is substantially pure. As used herein, "substantially pure" refers to material which is at least 50% pure (i.e., free from contaminants), at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure.

[0068] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer can be linear or branched, it can comprise modified amino acids, and it can be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. It is understood that, because the polypeptides of this invention are based upon antibodies, in certain embodiments, the polypeptides can occur as single chains or associated chains.

[0069] “Percent identity” refers to the extent of identity between two sequences (*e.g.*, amino acid sequences or nucleic acid sequences). Percent identity can be determined by aligning two sequences, introducing gaps to maximize identity between the sequences. Alignments can be generated using programs known in the art. For purposes herein, alignment of nucleotide sequences can be performed with the blastn program set at default parameters, and alignment of amino acid sequences can be performed with the blastp program set at default parameters (see National Center for Biotechnology Information (NCBI) on the worldwide web, ncbi.nlm.nih.gov).

[0070] As used herein, the term “host cell” can be any type of cell, *e.g.*, a primary cell, a cell in culture, or a cell from a cell line. In specific embodiments, the term “host cell” refers to a cell transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule, *e.g.*, due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

[0071] The term "overexpression" of B7-H4 in a particular tumor, tissue, or cell sample refers to B7-H4 (a B7-H4 polypeptide or a nucleic acid encoding such a polypeptide) that is present at a level higher than that which is present in non-diseased tissue or cells of the same type or origin or other cells in the proximity of a tumor or cancer. Such overexpression can be caused, for example, by mutation, gene amplification, increased transcription, or increased translation.

[0072] The term "primary" antibody or antigen-binding fragment thereof herein refers to an antibody or fragment that binds specifically to the target antigen in a sample. A primary antibody is generally the first antibody used in an immunohistochemical (IHC) procedure. In one embodiment, the primary antibody is the only antibody used in an IHC procedure. The term "secondary" antibody or antigen-binding fragment thereof herein refers to an antibody or fragment that binds specifically to a primary antibody, thereby forming a bridge between the primary antibody and a subsequent reagent, if any. The secondary antibody is generally the second antibody used in an immunohistochemical procedure. The term "tertiary antibody" herein refers to an antibody that binds specifically to a secondary antibody, thereby forming a bridge between the secondary antibody and a subsequent reagent, if any.

[0073] A "sample" of the present invention is of biological origin. In preferred embodiments, the sample is a human sample, but animal samples may also be used in the practice of the invention. Non-limiting sources of a sample for use in the present invention include solid tissue, biopsy, ascites, aspirates, fluidic extracts, blood (including circulating tumor cells), plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, tumors, organs, cell cultures and/or cell culture constituents, for example. A "cancerous sample" is a sample that contains a cancerous cell.

[0074] For the purposes herein, a "section" of a tissue sample refers to a single part or piece of a tissue sample, *e.g.*, a thin slice of tissue or cells cut from a tissue sample. It is understood that multiple sections of tissue samples may be taken and subjected to analysis according to the present invention. In some cases, the selected portion or section of tissue comprises a homogeneous population of cells. In other cases, the selected portion comprises a region of tissue, *e.g.*, the lumen as a non-limiting example. The selected portion can be as small as one cell or two cells, or could represent many thousands of cells, for example. In most cases, the collection of cells is important, and while the invention has been described for use in the detection of cellular components, the method may also be used for detecting non-cellular components of an organism (*e.g.*, soluble components in the blood as a non-limiting example).

[0075] The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label can be detectable by itself (*e.g.*, radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, can catalyze chemical alteration of a substrate compound or composition which is detectable.

[0076] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. The formulation can be sterile.

[0077] The terms “administer,, “administering,” “administration,, and the like, as used herein, refer to methods that may be used to enable delivery of a drug, *e.g.*, an anti-B7-H4 antibody or antigen-binding fragment thereof to the desired site of biological action (*e.g.*, intravenous administration). Administration techniques that can be employed with the agents and methods described herein are found in *e.g.*, Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, current edition, Pergamon; and Remington’s, *Pharmaceutical Sciences*, current edition, Mack Publishing Co., Easton, Pa.

[0078] As used herein, the terms “subject” and “patient” are used interchangeably. The subject can be an animal. In some embodiments, the subject is a mammal such as a non-human animal (*e.g.*, cow, pig, horse, cat, dog, rat, mouse, monkey or other primate, etc.). In some embodiments, the subject is a human.

[0079] The term "therapeutically effective amount" refers to an amount of a drug, *e.g.*, an anti-B7-H4 antibody or antigen-binding fragment thereof effective to treat a disease or disorder in a subject. In the case of cancer, the therapeutically effective amount of the drug can reduce the number of cancer cells; reduce the tumor size or burden; inhibit to some extent cancer cell infiltration into peripheral organs; inhibit to some extent tumor metastasis; inhibit, to some extent, tumor growth; relieve, to some extent one, or more of the symptoms associated with the cancer; and/or result in a favorable response such as increased progression-free survival (PFS), disease-free survival (DFS), overall survival (OS), complete response (CR), partial response (PR), or, in some cases, stable disease (SD), a decrease in progressive disease (PD), a reduced time to progression (TTP), or any combination thereof. To the extent the drug can prevent growth and/or kill existing cancer cells, it can be cytostatic and/or cytotoxic.

[0080] Terms such as "treating," "treatment," "to treat," "alleviating" and "to alleviate" refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a pathologic condition or disorder. Thus, those in need of treatment include those already diagnosed with or suspected of having the disorder. In certain embodiments, a subject is successfully "treated" for cancer according to the methods of the present invention if the patient shows one or more of the following: a reduction in the number of or complete absence of cancer cells; a reduction in the tumor size; inhibition or an absence of cancer cell infiltration into peripheral organs including, for example, the spread of cancer into soft tissue and bone; inhibition of or an absence of tumor metastasis; inhibition or an absence of tumor growth; relief of one or more symptoms associated with the specific cancer; reduced morbidity and mortality; improvement in quality of life; reduction in tumorigenicity, tumorigenic frequency, or tumorigenic capacity, of a tumor; reduction in the number or frequency of cancer stem cells in a tumor; differentiation of tumorigenic cells to a non-tumorigenic state; increased progression-free survival (PFS), disease-free survival (DFS), overall survival (OS), complete response (CR), partial response (PR), stable disease (SD), a decrease in progressive disease (PD), a reduced time to progression (TTP), or any combination thereof.

[0081] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals in which a population of cells are characterized by unregulated cell growth. Examples of cancer include, but are not limited to, gynecological cancers (*e.g.*,

breast cancer (including triple negative breast cancer, ductal carcinoma, ovarian cancer, and endometrial cancer), non-small cell lung cancer, pancreatic cancer, thyroid cancer, kidney cancer (*e.g.*, renal cell carcinoma) and bladder cancer (*e.g.*, urothelial cell carcinoma). The cancer can be a "cancer that expresses B7-H4" or a "B7-H4 expressing cancer." Such terms refer to a cancer comprising cells that express B7-H4. The cancer can be a solid tumor that expresses B7-H4. The cancer can be a primary tumor or may be advanced or metastatic cancer.

[0082] A "refractory" cancer is one that progresses even though an anti-tumor treatment, such as a chemotherapy, is administered to the cancer patient.

[0083] A "recurrent" cancer is one that has regrown, either at the initial site or at a distant site, after a response to initial therapy.

[0084] As used in the present disclosure and claims, the singular forms "a," "an," and "the" include plural forms unless the context clearly dictates otherwise.

[0085] It is understood that wherever embodiments are described herein with the language "comprising," otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are also provided. In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. patent law and can mean " includes," "including," and the like; "consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited are not changed by the presence of more than that which is recited, but excludes prior art embodiments

[0086] Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. The term "and/or" as used in a phrase such as "A and/or B" herein is intended to include both "A and B," "A or B," "A," and "B." Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0087] As used herein, the terms "about" and "approximately," when used to modify a numeric value or numeric range, indicate that deviations of 5% to 10% above and 5% to

10% below the value or range remain within the intended meaning of the recited value or range.

[0088] Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

Antibodies

[0089] In a specific aspect, provided herein are antibodies (e.g., monoclonal antibodies) and antigen-binding fragments thereof which specifically bind to B7-H4 (*e.g.*, human B7-H4). The amino acid sequences for human B7-H4 is known in the art and also provided herein as represented by SEQ ID NO:1.

Human B7-H4:

MASLGQILFWSIISIIIILAGAIALIIGFGISGRHSITVTTVASAGNIGEDGILSCTFEPD IKLSDIVIQWLKEGVLGLVHEFKEGKDELSEQDEMFRGRTAVFADQVIVGNASLR LKNVQLTDAGTYKCYIITSKGKGNANLEYKTGAFSMPEVNVDYNASSETLRCEA PRWFPQPTVVWASQVDQGANFSEVSNTSFELNSENVTMKVVSVLYNVTINNTYS CMIENDIAKATGDIKVTESEIKRRSHLQLLNSKASLCVSSFFAISWALLPLSPYLML K (SEQ ID NO:1)

[0090] In certain embodiments, an anti-B7-H4 antibody or antigen-binding fragment thereof is an antibody disclosed in Table 1 or is an antibody or antigen-binding fragment thereof comprising the complementarity determining regions (CDRs), variable heavy chain and/or variable light chain, and/or heavy chain and/or light chain of an antibody disclosed in Table 1.

Table 1: Exemplary anti-B7-H4 antibodies

Clone ID	Hybridoma VH/VL	Antibody Type	SEQ ID NOs				
			CDRs	VH	VL	H	L
J511	M6	mIgG1	22-26, 29	6	9	89	19
J512	M6	mIgG2a	22-26, 29	6	9	16	19
J517	M6 – De-N-gly (1)	mIgG2a	22-25, 27, 29	6	10	16	30
J518	M6	mIgG2a	22-25, 28,	6	11	16	31

	De-N-gly (2)		29				
J513	M11	mIgG1	22-26, 29	7	9	90	19
J514	M11	mIgG2a	22-26, 29	7	9	17	19
J519	M11 – De-N-gly (1)	mIgG2a	22-25, 27, 29	7	10	17	30
J520	M11 De-N-gly (2)	mIgG2a	22-25, 28, 29	7	11	17	31
J515	M15	mIgG1	22-26, 29	8	9	91	19
J516	M15	mIgG2a	22-26, 29	8	9	18	19
J521	M15 – De-N-gly (1)	mIgG2a	22-25, 27, 29	8	10	18	30
J522	M15 De-N-gly (2)	mIgG2a	22-25, 28, 29	8	11	18	31

[0091] In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4 and comprises six CDRs listed in Table 2, i.e., the six CDRs of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, a sequence selected from the group consisting of SEQ ID NOs:26-28, and SEQ ID NO:29.

Table 2: CDR Amino Acid Sequences

Description of CDR	**CDR Amino Acid Sequence (SEQ ID NO)**
VH-CDR1	GFSLSTYG (SEQ ID NO:22)
VH-CDR2	WWNDD (SEQ ID NO:23)
VH-CDR3	VDGYYWYFDV (SEQ ID NO:24)
VL-CDR1	RSSQSIVHSNRNTYLE (SEQ ID NO:25)
VL-CDR2	NVSNRFS (SEQ ID NO:26)
VL-CDR2, De-N-glycosylated (1)	NVANRFS (SEQ ID NO:27)
VL-CDR2, De-N-glycosylated (2)	QVSNRFS (SEQ ID NO:28)
VL-CDR3	FQGSHVPLT (SEQ ID NO:29)

[0092] In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4 and comprises a VH listed in Table 3, *e.g.*, in combination with a VL.

Table 3: Variable Heavy Chain (VH) Amino Acid Sequences

Description of VH	VH Amino Acid Sequence (SEQ ID NO)
M6 VH (J511, J512, J517, J518)	QVTLKESGPGILQPSQTLSLTCSFSGFSLSTYGLGVGWIRQP SGKGLDWLANIWWNDDKYYNSALKSRLTISKDTSNNQVF LKISSVDTADTGTYYCAQVDGYYWYFDVWGAGTTVTVSS (SEQ ID NO:6)
M11 VH (J513, J514, J519, J520)	QVTLKESGPGILQPSQTLSLTCSLSGFSLSTYGLGVGWIRQP SGKGLGWLANIWWNDDKYYNSALKSRLTISKDTSNNQVF LKISSVDTADTGTYYCAQVDGYYWYFDVWGAGTTVTVSS (SEQ ID NO:7)
M15 VH (J515, J516, J521, J522)	QVTLKESGPGILQSSQTLSLTCSFSGFSLSTYGLGVGWIRQP SGKGLDWLANIWWNDDKYYNSALKSRLTISKDTSNNQVF LKISSVDTADTGTYYCAQVDGYYWYFDVWGAGTTVTVSS (SEQ ID NO:8)

[0093] In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4 and comprises a VL listed in Table 4, *e.g.*, in combination with a VH.

Table 4: Variable Light Chain (VL) Amino Acid Sequences

Description of VL	VL Amino Acid Sequence (SEQ ID NO)
M6, M11, M15 VL (J511-J516)	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNRNTYLEWY LQKPGQSPKLLIYNVSNRFSGVPDRFSGSGSGTDFTLKISR VEAEDLGVYYCFQGSHVPLTFGAGTKLELK (SEQ ID NO:9)
De-N-glycosylated VL (1) (J517, J519, J521)	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNRNTYLEWY LQKPGQSPKLLIYNVANRFSGVPDRFSGSGSGTDFTLKISR VEAEDLGVYYCFQGSHVPLTFGAGTKLELK (SEQ ID NO:10)
De-N-glycosylated VL (2) (J518, J520, J522)	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNRNTYLEWY LQKPGQSPKLLIYQVSNRFSGVPDRFSGSGSGTDFTLKISR VEAEDLGVYYCFQGSHVPLTFGAGTKLELK (SEQ ID NO:11)

[0094] In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4 and comprises a VH listed in Table 3 and a VL of listed in Table 4. In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4 and comprises a

VH comprising or consisting of a sequence listed in Table 3 and a VL comprising or consisting of listed in Table 4.

[0095] In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH comprising a sequence at least 80% identical to a VH sequence in Table 3 and a VL comprising a sequence at least 80% identical to a VL sequence in Table 4. In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH comprising a sequence at least 85% identical to a VH sequence in Table 3 and a VL comprising a sequence at least 85% identical to a VL sequence in Table 4.

[0096] In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH consisting of a sequence at least 80% identical to a VH sequence in Table 3 and a VL consisting of a sequence at least 80% identical to a VL sequence in Table 4. In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH consisting of a sequence at least 85% identical to a VH sequence in Table 3 and a VL consisting of a sequence at least 85% identical to a VL sequence in Table 4.

[0097] In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH comprising a sequence at least 90% identical to a VH sequence in Table 3 and a VL comprising a sequence at least 90% identical to a VL sequence in Table 4. In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH comprising a sequence at least 95% identical to a VH sequence in Table 3 and a VL comprising a sequence at least 95% identical to a VL sequence in Table 4.

[0098] In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH comprising a sequence at least 96% identical to a VH sequence in Table 3 and a VL comprising a sequence at least 96% identical to a VL sequence in Table 4. In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH comprising a sequence at least 97% identical to a VH

sequence in Table 3 and a VL comprising a sequence at least 97% identical to a VL sequence in Table 4. In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH comprising a sequence at least 98% identical to a VH sequence in Table 3 and a VL comprising a sequence at least 98% identical to a VL sequence in Table 4. In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH comprising a sequence at least 99% identical to a VH sequence in Table 3 and a VL comprising a sequence at least 99% identical to a VL sequence in Table 4.

[0099] In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH consisting of a sequence at least 96% identical to a VH sequence in Table 3 and a VL consisting of a sequence at least 96% identical to a VL sequence in Table 4. In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH consisting of a sequence at least 97% identical to a VH sequence in Table 3 and a VL consisting of a sequence at least 97% identical to a VL sequence in Table 4. In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH consisting of a sequence at least 98% identical to a VH sequence in Table 3 and a VL consisting of a sequence at least 98% identical to a VL sequence in Table 4. In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4, comprises a VH consisting of a sequence at least 99% identical to a VH sequence in Table 3 and a VL consisting of a sequence at least 99% identical to a VL sequence in Table 4.

[0100] In certain aspects, an antibody or antigen-binding fragment thereof described herein is described by its VL domain alone, its VH domain alone, its 3 VL CDRs alone, or its 3 VH CDRs alone. *See*, for example, Rader C *et al.*, (1998) PNAS 95: 8910-8915, which is incorporated herein by reference in its entirety, describing the humanization of the mouse anti-αvβ3 antibody by identifying a complementing light chain or heavy chain, respectively, from a human light chain or heavy chain library, resulting in humanized antibody variants having affinities as high or higher than the affinity of the original antibody. *See also* Clackson T *et al.*, (1991) Nature 352: 624-628, which is incorporated

herein by reference in its entirety, describing methods of producing antibodies that specifically bind a specific antigen by using a specific VL domain (or VH domain) and screening a library for the complementary VH domain (or VL domain). The screen produced 14 new partners for a specific VH domain and 13 new partners for a specific VL domain, which were strong binders, as determined by ELISA. *See also* Kim SJ & Hong HJ, (2007) J Microbiol 45: 572-577, which is incorporated herein by reference in its entirety, describing methods of producing antibodies that specifically bind a specific antigen by using a specific VH domain and screening a library (*e.g.*, human VL library) for complementary VL domains; the selected VL domains in turn could be used to guide selection of additional complementary (*e.g.*, human) VH domains.

[0101] In certain aspects, the CDRs of an antibody or antigen-binding fragment thereof can be determined according to the Chothia numbering scheme, which refers to the location of immunoglobulin structural loops (*see*, *e.g.*, Chothia C & Lesk AM, (1987), J Mol Biol 196: 901-917; Al-Lazikani B *et al.*, (1997) J Mol Biol 273: 927-948; Chothia C *et al.*, (1992) J Mol Biol 227: 799-817; Tramontano A *et al.*, (1990) J Mol Biol 215(1): 175-82; and U.S. Patent No. 7,709,226). Typically, when using the Kabat numbering convention, the Chothia CDR-H1 loop is present at heavy chain amino acids 26 to 32, 33, or 34, the Chothia CDR-H2 loop is present at heavy chain amino acids 52 to 56, and the Chothia CDR-H3 loop is present at heavy chain amino acids 95 to 102, while the Chothia CDR-L1 loop is present at light chain amino acids 24 to 34, the Chothia CDR-L2 loop is present at light chain amino acids 50 to 56, and the Chothia CDR-L3 loop is present at light chain amino acids 89 to 97. The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34).

[0102] In certain aspects, provided herein are antibodies and antigen-binding fragments thereof that specifically bind to B7-H4 (*e.g.*, human B7-H4) and comprise the Chothia VH and VL CDRs of an antibody. In certain embodiments, antibodies or antigen-binding fragments thereof that specifically bind to B7-H4 (*e.g.*, human B7-H4) and comprise one or more CDRs, in which the Chothia and Kabat CDRs have the same amino acid sequence. In certain embodiments, provided herein are antibodies and antigen-binding

fragments thereof that specifically bind to B7-H4 (*e.g.*, human B7-H4) and comprise combinations of Kabat CDRs and Chothia CDRs.

[0103] In certain aspects, the CDRs of an antibody or antigen-binding fragment thereof can be determined according to the IMGT numbering system as described in Lefranc M-P, (1999) The Immunologist 7: 132-136 and Lefranc M-P *et al.*, (1999) Nucleic Acids Res 27: 209-212. According to the IMGT numbering scheme, VH-CDR1 is at positions 26 to 35, VH-CDR2 is at positions 51 to 57, VH-CDR3 is at positions 93 to 102, VL-CDR1 is at positions 27 to 32, VL-CDR2 is at positions 50 to 52, and VL-CDR3 is at positions 89 to 97. In a particular embodiment, provided herein are antibodies and antigen-binding fragments thereof that specifically bind to B7-H4 (*e.g.*, human B7-H4) and comprise the IMGT VH and VL CDRs of an antibody listed in Tables 3 and 4, for example, as described in Lefranc M-P (1999) *supra* and Lefranc M-P *et al.*, (1999) *supra*).

[0104] In certain aspects, the CDRs of an antibody or antigen-binding fragment thereof can be determined according to MacCallum RM *et al.*, (1996) J Mol Biol 262: 732-745. *See also*, *e.g.*, Martin A. "Protein Sequence and Structure Analysis of Antibody Variable Domains," in *Antibody Engineering*, Kontermann and Dübel, eds., Chapter 31, pp. 422-439, Springer-Verlag, Berlin (2001). In a particular embodiment, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to B7-H4 (*e.g.*, human B7-H4) are determined by the method in MacCallum RM *et al.*

[0105] In certain aspects, the CDRs of an antibody or antigen-binding fragment thereof can be determined according to the AbM numbering scheme, which refers AbM hypervariable regions which represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software (Oxford Molecular Group, Inc.). In a particular embodiment, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to B7-H4 (*e.g.*, human B7-H4) are determined by the AbM numbering scheme.

[0106] In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4 and comprises a heavy chain listed in Table 5, *e.g.*, in combination with a light chain.

Table 5: Heavy Chain Amino Acid Sequences

Description of Heavy Chain	Heavy Chain Amino Acid Sequence (SEQ ID NO)

M6 H (J511)	QVTLKESGPGILQPSQTLSLTCSFSGFSLSTYGLGVGWIRQP SGKGLDWLANIWWNDDKYYNSALKSRLTISKDTSNNQVF LKISSVDTADTGTYYCAQVDGYYWYFDVWGAGTTVTVSS AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVT WNSGSLSSGVHTFPAVLESDLYTLSSSVTVPSSPRPSETVTC NVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKP KDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTA QTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAA FPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMI TDFFPEDITVEWQWNGQPAENYKNTQPIMNTNGSYFVYSK LNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK (SEQ ID NO:89)
M6 H (J512, J517, J518)	QVTLKESGPGILQPSQTLSLTCSFSGFSLSTYGLGVGWIRQP SGKGLDWLANIWWNDDKYYNSALKSRLTISKDTSNNQVF LKISSVDTADTGTYYCAQVDGYYWYFDVWGAGTTVTVSS AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVT WNSGSLSSGVHTFPAVLESDLYTLSSSVTVPSSPRPSETVTC NVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKI KDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTA QTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNK DLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTC MVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFM YSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPG K (SEQ ID NO:16)
M11 H (J513)	QVTLKESGPGILQPSQTLSLTCSLSGFSLSTYGLGVGWIRQP SGKGLGWLANIWWNDDKYYNSALKSRLTISKDTSNNQVF LKISSVDTADTGTYYCAQVDGYYWYFDVWGAGTTVTVSS AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVT WNSGSLSSGVHTFPAVLESDLYTLSSSVTVPSSPRPSETVTC NVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKP KDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTA QTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAA FPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMI TDFFPEDITVEWQWNGQPAENYKNTQPIMNTNGSYFVYSK LNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK (SEQ ID NO:90)
M11 H (J514, J519, J520)	QVTLKESGPGILQPSQTLSLTCSLSGFSLSTYGLGVGWIRQP SGKGLGWLANIWWNDDKYYNSALKSRLTISKDTSNNQVF LKISSVDTADTGTYYCAQVDGYYWYFDVWGAGTTVTVSS AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVT WNSGSLSSGVHTFPAVLESDLYTLSSSVTVPSSPRPSETVTC NVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKI KDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTA QTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNK DLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTC MVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFM YSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPG K (SEQ ID NO:17)

M15 H (J515)	QVTLKESGPGILQSSQTLSLTCSFSGFSLSTYGLGVGWIRQP SGKGLDWLANIWWNDDKYYNSALKSRLTISKDTSNNQVF LKISSVDTADTGTYYCAQVDGYYWYFDVWGAGTTVTVSS AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVT WNSGSLSSGVHTFPAVLESDLYTLSSSVTVPSSPRPSETVTC NVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKP KDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTA QTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAA FPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMI TDFFPEDITVEWQWNGQPAENYKNTQPIMNTNGSYFVYSK LNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK (SEQ ID NO:91)
M15 H (J516, J521, J522)	QVTLKESGPGILQSSQTLSLTCSFSGFSLSTYGLGVGWIRQP SGKGLDWLANIWWNDDKYYNSALKSRLTISKDTSNNQVF LKISSVDTADTGTYYCAQVDGYYWYFDVWGAGTTVTVSS AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVT WNSGSLSSGVHTFPAVLESDLYTLSSSVTVPSSPRPSETVTC NVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKI KDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTA QTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNK DLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTC MVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFM YSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPG K (SEQ ID NO:18)

[0107] In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4 and comprises a light chain listed in Table 6, *e.g.*, in combination with a heavy chain.

Table 6: Light Chain Amino Acid Sequences

Description of Light Chain	Light Chain Amino Acid Sequence (SEQ ID NO)
M6, M11, M15 L (J511 to J516)	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNRNTYLEWY LQKPGQSPKLLIYNVSNRFSGVPDRFSGSGSGTDFTLKISRV EAEDLGVYYCFQGSHVPLTFGAGTKLELKRADAAPTVSIFP PSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGV LNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHK TSTSPIVKSFNRNEC (SEQ ID NO:19)
De-N-glycosylated L (1) (J517, J519, J521)	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNRNTYLEWY LQKPGQSPKLLIYNVANRFSGVPDRFSGSGSGTDFTLKISR VEAEDLGVYYCFQGSHVPLTFGAGTKLELKRADAAPTVSI FPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNG VLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATH KTSTSPIVKSFNRNEC (SEQ ID NO:30)
De-N-glycosylated L (2) (J518, J520, J522)	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNRNTYLEWY LQKPGQSPKLLIYQVSNRFSGVPDRFSGSGSGTDFTLKISRV EAEDLGVYYCFQGSHVPLTFGAGTKLELKRADAAPTVSIFP

	PSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGV LNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHK TSTSPIVKSFNRNEC (SEQ ID NO:31)

[0108] In certain embodiments, an antibody or antigen-binding fragment thereof described herein specifically binds to human B7-H4 and comprises a heavy chain listed in Table 5 and a light chain listed in Table 6.

[0109] In specific aspects, provided herein are antibodies that comprise a heavy chain and a light chain. With respect to the heavy chain, in a specific embodiment, the heavy chain of an antibody described herein can be an alpha (α), delta (δ), epsilon (ε), gamma (γ) or mu (μ) heavy chain. In a particular embodiment, an antibody described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4), comprises a heavy chain wherein the amino acid sequence of the VH domain comprises an amino acid sequence set forth in Table 3 and wherein the constant region of the heavy chain comprises the amino acid sequence of a murine IgG2a heavy chain constant region.

[0110] In a particular embodiment, an antibody described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4), comprises a heavy chain wherein the amino acid sequence of the VH domain comprises an amino acid sequence set forth in Table 3 and wherein the constant region of the heavy chain comprises the amino acid sequence of SEQ ID NO:87:

AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLES DLYTLSSSVTVPSSPRPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFP PKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVS ALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQV TLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVER NSYSCSVVHEGLHNHHTTKSFSRTPGK (SEQ ID NO:87)

[0111] In a particular embodiment, an antibody described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4), comprises a heavy chain wherein the amino acid sequence of the VH domain comprises an amino acid sequence set forth in Table 3 and wherein the constant region of the heavy chain comprises the amino acid sequence of a murine IgG1 heavy chain constant region.

[0112] In a particular embodiment, an antibody described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4), comprises a heavy chain wherein the amino acid sequence of the VH domain comprises an amino acid sequence

set forth in Table 3 and wherein the constant region of the heavy chain comprises the amino acid sequence of SEQ ID NO:92:

AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLES
DLYTLSSSVTVPSSPRPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFP
PKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVS
ELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVS
LTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMNTNGSYFVYSKLNVQKSNWEAGNT
FTCSVLHEGLHNHHTEKSLSHSPGK (SEQ ID NO:92)

[0113] With respect to the light chain, in a specific embodiment, the light chain of an antibody described herein is a kappa light chain or a lamda light chain. In a particular embodiment, an antibody described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4), comprises a light chain wherein the amino acid sequence of the VL domain comprises an amino acid sequence set forth in Table 4 and wherein the constant region of the heavy chain comprises the amino acid sequence of SEQ ID NO:88:

RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQ
DSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC (SEQ ID NO:88).

[0114] In another specific embodiment, an antibody described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4) comprises (i) a heavy chain wherein the amino acid sequence of the VH domain comprises an amino acid sequence set forth in Table 3 and wherein the constant region of the heavy chain comprises the amino acid sequence of SEQ ID NO:87 and (ii) a light chain wherein the amino acid sequence of the VL domain comprises an amino acid sequence set forth in Table 4 and wherein the constant region of the heavy chain comprises the amino acid sequence of SEQ ID NO:88.

[0115] In another specific embodiment, an antibody described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4) comprises (i) a heavy chain wherein the amino acid sequence of the VH domain comprises an amino acid sequence set forth in Table 3 and wherein the constant region of the heavy chain comprises the amino acid sequence of SEQ ID NO:92 and (ii) a light chain wherein the amino acid sequence of the VL domain comprises an amino acid sequence set forth in Table 4 and wherein the constant region of the heavy chain comprises the amino acid sequence of SEQ ID NO:88.

[0116] In a specific embodiment, an antibody described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4) comprises a VH domain and a VL domain comprising any amino acid sequence described herein, and wherein the constant regions comprise the amino acid sequences of the constant regions of an IgG, IgE, IgM, IgD, IgA, or IgY immunoglobulin molecule, or a murine IgG, IgE, IgM, IgD, or IgA immunoglobulin molecule. In another specific embodiment, an antibody described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4) comprises a VH domain and a VL domain comprising any amino acid sequence described herein, and wherein the constant regions comprise the amino acid sequences of the constant regions of an IgG, IgE, IgM, IgD, IgA, or IgY immunoglobulin molecule, any class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2), or any subclass (*e.g.*, IgG_{2a} and IgG_{2b}) of immunoglobulin molecule. In a particular embodiment, the constant regions comprise the amino acid sequences of the constant regions of a murine IgG, IgE, IgM, IgD, or IgA, immunoglobulin molecule, any class (*e.g.*, IgG_1, IgG_2, and IgG_3,), or any subclass (*e.g.*, IgG_{2a} and IgG_{2b}) of immunoglobulin molecule. In a particular embodiment, an antibody described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4) comprises a VH domain and a VL domain comprising any amino acid sequence described herein and murine constant domains. In a particular embodiment, an antibody described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4) comprises a VH domain and a VL domain comprising any amino acid sequence described herein and rabbit constant domains.

[0117] In specific embodiments, an antibody or antigen-binding fragment thereof comprises the VH and VL sequences of the amino acid sequences of SEQ ID NOs:6 and 9, respectively. In specific embodiments, the antibody or antigen-binding fragment thereof in an engineered variant of an antibody or antigen-binding fragment thereof comprising the VH and VL sequences of the amino acid sequences of SEQ ID NOs:6 and 9, wherein the engineering removes one or more glycosylation sites, *e.g.*, in one or more CDRs (*e.g.*, VL-CDR2). In specific embodiments, an antibody or antigen-binding fragment thereof comprises the VH and VL sequences of the amino acid sequences of SEQ ID NOs:6 and 10, respectively. In specific embodiments, an antibody or antigen-binding fragment thereof comprises the VH and VL sequences of the amino acid sequences of SEQ ID NOs:6 and 11, respectively. In specific embodiments, the antibody

or antigen-binding fragment thereof (*e.g.*, an antibody or antigen-binding fragment thereof comprising a VH of SEQ ID NO:6 and a VL of SEQ ID NO:9, 10, or 11) further comprises the constant domain of a murine IgG_1 heavy chain (*e.g.*, J511). In specific embodiments, the antibody or antigen-binding fragment thereof (*e.g.*, an antibody or antigen-binding fragment thereof comprising a VH of SEQ ID NO:6 and a VL of SEQ ID NO:9, 10, or 11) further comprises the constant domain of a murine IgG_{2a} heavy chain (*e.g.*, J512).

[0118] In specific embodiments, an antibody or antigen-binding fragment thereof comprises the VH and VL sequences of the amino acid sequences of SEQ ID NOs:7 and 9, respectively. In specific embodiments, the antibody or antigen-binding fragment thereof in an engineered variant of an antibody or antigen-binding fragment thereof comprising the VH and VL sequences of the amino acid sequences of SEQ ID NOs:7 and 9, wherein the engineering removes one or more glycosylation sites, *e.g.*, in one or more CDRs (*e.g.*, VL-CDR2). In specific embodiments, an antibody or antigen-binding fragment thereof comprises the VH and VL sequences of the amino acid sequences of SEQ ID NOs:7 and 10, respectively. In specific embodiments, an antibody or antigen-binding fragment thereof comprises the VH and VL sequences of the amino acid sequences of SEQ ID NOs:7 and 11, respectively. In specific embodiments, the antibody or antigen-binding fragment thereof (*e.g.*, an antibody or antigen-binding fragment thereof comprising a VH of SEQ ID NO:7 and a VL of SEQ ID NO:9, 10, or 11) further comprises the constant domain of a murine IgG_1 heavy chain (*e.g.*, J513). In specific embodiments, the antibody or antigen-binding fragment thereof (*e.g.*, an antibody or antigen-binding fragment thereof comprising a VH of SEQ ID NO:7 and a VL of SEQ ID NO:9, 10, or 11) further comprises the constant domain of a murine IgG_{2a} heavy chain (*e.g.*, J514).

[0119] In specific embodiments, an antibody or antigen-binding fragment thereof comprises the VH and VL sequences of the amino acid sequences of SEQ ID NOs:8 and 9, respectively. In specific embodiments, the antibody or antigen-binding fragment thereof in an engineered variant of an antibody or antigen-binding fragment thereof comprising the VH and VL sequences of the amino acid sequences of SEQ ID NOs:8 and 9, wherein the engineering removes one or more glycosylation sites, *e.g.*, in one or more CDRs (*e.g.*, VL-CDR2). In specific embodiments, an antibody or antigen-binding

fragment thereof comprises the VH and VL sequences of the amino acid sequences of SEQ ID NOs:8 and 10, respectively. In specific embodiments, an antibody or antigen-binding fragment thereof comprises the VH and VL sequences of the amino acid sequences of SEQ ID NOs:8 and 11, respectively. In specific embodiments, the antibody or antigen-binding fragment thereof (*e.g.*, an antibody or antigen-binding fragment thereof comprising a VH of SEQ ID NO:8 and a VL of SEQ ID NO:9, 10, or 11) further comprises the constant domain of a murine IgG_1 heavy chain (*e.g.*, J515). In specific embodiments, the antibody or antigen-binding fragment thereof (*e.g.*, an antibody or antigen-binding fragment thereof comprising a VH of SEQ ID NO:8 and a VL of SEQ ID NO:9, 10, or 11) further comprises the constant domain of a murine IgG_{2a} heavy chain (*e.g.*, J516).

[0120] In specific embodiments, an antibody or antigen-binding fragment thereof (i) comprises the CDR sequences of M6 (*e.g.*, the amino acid sequences of SEQ ID NOs:22-26 and 29), (ii) the VH and VL sequences of M6 or a De-N-glycosylated variant thereof (*e.g.*, a VH comprising the amino acid sequence of SEQ ID NO:6 and a VL comprising the amino acid sequence of SEQ ID NO:9, 10, or 11), (iii) the heavy and light chain sequences of murine IgG2a M6 or a De-N-glycosylated variant thereof (*e.g.*, a heavy chain comprising the amino acid sequence of SEQ ID NO:16 and a light chain comprising the amino acid sequence of SEQ ID NO:19, 30, or 31) or (iv) the heavy and light chain sequences of murine IgG1 M6 (*e.g.*, a heavy chain comprising the amino acid sequence of SEQ ID NO:89 and a light chain comprising the amino acid sequence of SEQ ID NO:19).

[0121] In specific embodiments, an antibody or antigen-binding fragment thereof (i) comprises the CDR sequences of M11 (*e.g.*, the amino acid sequences of SEQ ID NOs:22-25, 27, and 29), (ii) the VH and VL sequences of M11 or a De-N-glycosylated variant thereof (*e.g.*, a VH comprising the amino acid sequence of SEQ ID NO:7 and a VL comprising the amino acid sequence of SEQ ID NO:9, 10, or 11), (iii) the heavy and light chain sequences of murine IgG2a M11 or a De-N-glycosylated variant thereof (*e.g.*, a heavy chain comprising the amino acid sequence of SEQ ID NO:17 and a light chain comprising the amino acid sequence of SEQ ID NO:19, 30, or 31), or (iv) the heavy and light chain sequences of murine IgG1 M11 (*e.g.*, a heavy chain comprising the amino acid sequence of SEQ ID NO:90 and a light chain comprising the amino acid sequence of SEQ ID NO:19).

[0122] In specific embodiments, an antibody or antigen-binding fragment thereof (i) comprises the CDR sequences of M15 (*e.g.*, the amino acid sequences of SEQ ID NOs:22-25, 28, and 29), (ii) the VH and VL sequences of M15 or a De-N-glycosylated variant thereof (*e.g.*, a VH comprising the amino acid sequence of SEQ ID NO:8 and a VL comprising the amino acid sequence of SEQ ID NO:9, 10, or 11), (iii) the heavy and light chain sequences of murine IgG2a M15 or a De-N-glycosylated variant thereof (*e.g.*, a heavy chain comprising the amino acid sequence of SEQ ID NO:18 and a light chain comprising the amino acid sequence of SEQ ID NO:19, 30, or 31) or (iv) the heavy and light chain sequences of murine IgG1 M15 (*e.g.*, a heavy chain comprising the amino acid sequence of SEQ ID NO:91 and a light chain comprising the amino acid sequence of SEQ ID NO:19).

[0123] In another particular embodiment, an antibody or antigen-binding fragment thereof described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4), comprises a heavy chain and a light chain, wherein (i) the heavy chain comprises a VH domain comprising the VH CDR1, VH CDR2, and VH CDR3 amino acid sequences of an antibody listed in Table 1 (*e.g.*, SEQ ID NOs:22-24); (ii) the light chain comprises a VL domain comprising the VL CDR1, VL CDR2, and VL CDR3 amino acid sequences of the same antibody listed in Table 1 (*e.g.*, SEQ ID NOs:25, 26, and 29, 25, 27, and 29, or 25, 28, and 29); (iii) the heavy chain further comprises a murine IgG1 constant domain (*e.g.* comprising the amino acid sequence of SEQ ID NO:92) or a murine IgG2a constant domain (*e.g.* comprising the amino acid sequence of SEQ ID NO:87); and (iv) the light chain further comprises a murine kappa constant domain (*e.g.*, comprising the amino acid sequence of SEQ ID NO:88).

[0124] In another particular embodiment, an antibody or antigen-binding fragment thereof described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4), comprises a heavy chain and a light chain, wherein (i) the heavy chain comprises a VH domain comprising the amino acid sequence of an antibody listed in Table 1 (*e.g.*, SEQ ID NOs:6-8); (ii) the light chain comprises a VL domain comprising the amino acid sequence of the same antibody listed in Table 1 (*e.g.*, SEQ ID NOs:9-11); (iii) the heavy chain further comprises a murine IgG1 constant domain (*e.g.* comprising the amino acid sequence of SEQ ID NO:92) or a murine IgG2a constant domain (*e.g.* comprising the amino acid sequence of SEQ ID NO:87); and (iv) the light chain further comprises a

murine kappa constant domain (*e.g.*, comprising the amino acid sequence of SEQ ID NO:88).

[0125] In certain embodiments, an antibody or antigen-binding fragment thereof described herein, which specifically binds to B7-H4 (*e.g.*, human B7-H4), comprises one, two, or more VH framework regions (FRs) having the amino acid sequences described herein for an antibody set forth in Table 7 (*e.g.*, SEQ ID NOs:38-49). In some embodiments, an antibody or antigen-binding fragment thereof described herein, which specifically binds to B7-H4 (*e.g.*, human B7-H4), comprises one, two, or more VL framework regions (FRs) having the amino acid sequences described herein for an antibody set forth in Table 7 (*e.g.*, SEQ ID NOs:50-53). In specific embodiments, an antibody or antigen-binding fragment thereof described herein, which specifically binds to B7-H4 (*e.g.*, human B7-H4), comprises one, two, or more VH framework regions having the amino acid sequences described herein for an antibody set forth in Table 7, *supra*, and one, two, or more VL framework regions having the amino acid sequences described herein for the same antibody set forth in Table 7 (*e.g.*, SEQ ID NOs:38-41 and 50-53; SEQ ID NOs:42-45 and 50-53; or SEQ ID NOs:46-49 and 50-53).

Table 7: Framework Amino Acid Sequences

Hybridoma VH/VL	FR1 (SEQ ID NO)	FR2 (SEQ ID NO)	FR3 (SEQ ID NO)	FR4 (SEQ ID NO)
M6 VH	QVTLKESGP GILQPSQTLS LTCSFS (SEQ ID NO:38)	GVGWIRQPSG KGLDWLANI (SEQ ID NO:39)	KYYNSALKSRLTISKD TSNNQVFLKISSVDTA DTGTYYCAQ (SEQ ID NO:40)	WGAGTTVTV SS (SEQ ID NO:41)
M11 VH	QVTLKESGP GILQPSQTLS LTCSLS (SEQ ID NO:42)	GVGWIRQPSG KGLGWLANI (SEQ ID NO:43)	KYYNSALKSRLTISKD TSNNQVFLKISSVDTA DTGTYYCAQ (SEQ ID NO:44)	WGAGTTVTV SS (SEQ ID NO:45)
M15 VH	QVTLKESGP GILQSSQTLS LTCSFS (SEQ ID NO:46)	GVGWIRQPSG KGLDWLANI (SEQ ID NO:47)	KYYNSALKSRLTISKD TSNNQVFLKISSVDTA DTGTYYCAQ (SEQ ID NO:48)	WGAGTTVTV SS (SEQ ID NO:49)
M6, M11, M15 VL	DVLMTQTPL SLPVSLGDQ ASISC (SEQ ID NO:50)	YLQKPGQSPK LLIY (SEQ ID NO:51)	GVPDRFSGSGSGTDFT LKISRVEAEDLGVYY C (SEQ ID NO:52)	FGAGTKLELK (SEQ ID NO:53)

[0126] In certain embodiments, an antibody or antigen-binding fragment thereof described herein, which specifically binds to B7-H4 (*e.g.*, human B7-H4), comprises VH framework regions (FRs) having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% sequence identity to the VH framework regions described herein in Table 7. In certain embodiments, an antibody or antigen-binding fragment thereof described herein, which specifically binds to B7-H4 (*e.g.*, human B7-H4), comprises VL framework regions (FRs) having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% sequence identity to the VL framework regions described herein Table 7. In some embodiments, an antibody or antigen-binding fragment thereof described herein, which specifically binds to B7-H4 (*e.g.*, human B7-H4), comprises VH framework regions (FRs) having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% sequence identity to the VH framework regions described herein Table 7, *supra*, and VL framework regions (FRs) having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% sequence identity to the VL framework regions described herein Table 7.

[0127] In another aspect, provided herein are antibodies or antigen-binding fragments thereof that bind the same epitope of B7-H4 (*e.g.*, an epitope of human B7-H4) as an antibody or antigen-binding fragment thereof described herein (*e.g.*, J511-J522).

[0128] Competition binding assays can be used to determine whether two antibodies bind to overlapping epitopes. Competitive binding can be determined in an assay in which the immunoglobulin under test inhibits specific binding of a reference antibody to a common antigen, such as B7-H4. Numerous types of competitive binding assays are known, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (*see* Stahli C *et al.*, (1983) Methods Enzymol 9: 242-253); solid phase direct biotin-avidin EIA (*see* Kirkland TN *et al.*, (1986) J Immunol 137: 3614-9); solid phase direct labeled assay, solid phase direct labeled sandwich assay (*see* Harlow E & Lane D, (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Press); solid phase direct label RIA using I-125 label (*see* Morel GA *et al.*, (1988) Mol Immunol 25(1): 7-15); solid phase direct biotin-avidin EIA (Cheung RC *et al.*, (1990) Virology 176: 546-52); and direct labeled RIA. (Moldenhauer

G *et al.*, (1990) Scand J Immunol 32: 77-82). Typically, such an assay involves the use of purified antigen (*e.g.*, B7-H4 such as human B7-H4) bound to a solid surface or cells bearing either of these, an unlabeled test immunoglobulin and a labeled reference immunoglobulin. Competitive inhibition can be measured by determining the amount of label bound to the solid surface or cells in the presence of the test immunoglobulin. Usually the test immunoglobulin is present in excess. Usually, when a competing antibody is present in excess, it will inhibit specific binding of a reference antibody to a common antigen by at least 50-55%, 55-60%, 60-65%, 65-70%, 70-75% or more. A competition binding assay can be configured in a large number of different formats using either labeled antigen or labeled antibody. In a common version of this assay, the antigen is immobilized on a 96-well plate. The ability of unlabeled antibodies to block the binding of labeled antibodies to the antigen is then measured using radioactive or enzyme labels. For further details *see*, for example, Wagener C *et al.*, (1983) J Immunol 130: 2308-2315; Wagener C *et al.*, (1984) J Immunol Methods 68: 269-274; Kuroki M *et al.*, (1990) Cancer Res 50: 4872-4879; Kuroki M *et al.*, (1992) Immunol Invest 21: 523-538; Kuroki M *et al.*, (1992) Hybridoma 11: 391-407 and Antibodies: A Laboratory Manual, Ed Harlow E & Lane D editors *supra*, pp. 386-389.

[0129] In one embodiment, a competition assay is performed using surface plasmon resonance (BIAcore®), *e.g.*, by an 'in tandem approach' such as that described by Abdiche YN *et al.*, (2009) Analytical Biochem 386: 172-180, whereby B7-H4 antigen is immobilized on the chip surface, for example, a CM5 sensor chip and the anti-B7-H4 antibodies are then run over the chip. To determine if an antibody or antigen-binding fragment thereof competes with an anti-B7-H4 antibody described herein, the anti-B7-H4 antibody is first run over the chip surface to achieve saturation and then the potential, competing antibody is added. Binding of the competing antibody or antigen-binding fragment thereof can then be determined and quantified relative to a non-competing control.

[0130] In one embodiment, Fortebio Octet competition binding is used to determine that a B7-H4 antibody or antigen-binding fragment thereof competitively inhibits the binding of another B7-H4 antibody or antigen-binding fragment thereof to B7-H4.

[0131] In another aspect, provided herein are antibodies that competitively inhibit (*e.g.*, in a dose dependent manner) an antibody or antigen-binding fragment thereof described

herein (*e.g.*, J511-J522) from binding to B7-H4 (*e.g.*, human B7-H4), as determined using assays known to one of skill in the art or described herein (*e.g.*, ELISA competitive assays, or suspension array or surface plasmon resonance assay).

[0132] In specific aspects, provided herein is an antibody or antigen-binding fragment which competitively inhibits (*e.g.*, in a dose dependent manner) binding to B7-H4 (*e.g.*, human B7-H4), of an antibody comprising a VH domain having the amino acid sequence set forth in SEQ ID NO:6, and a VL domain having the amino acid sequence set for the in SEQ ID NO:9.

[0133] In specific aspects, provided herein is an antibody or antigen-binding fragment which competitively inhibits (*e.g.*, in a dose dependent manner) binding to B7-H4 (*e.g.*, human B7-H4), of an antibody comprising a VH domain having the amino acid sequence set forth in SEQ ID NO:7, and a VL domain having the amino acid sequence set for the in SEQ ID NO:9.

[0134] In specific aspects, provided herein is an antibody or antigen-binding fragment which competitively inhibits (*e.g.*, in a dose dependent manner) binding to B7-H4 (*e.g.*, human B7-H4), of an antibody comprising a VH domain having the amino acid sequence set forth in SEQ ID NO:8, and a VL domain having the amino acid sequence set for the in SEQ ID NO:9.

[0135] In specific aspects, provided herein is an antibody or antigen-binding fragment which competitively inhibits (*e.g.*, in a dose dependent manner) binding to B7-H4 (*e.g.*, human B7-H4), of an antibody comprising a VH domain having the amino acid sequence set forth in SEQ ID NO:6, and a VL domain having the amino acid sequence set for the in SEQ ID NO:10.

[0136] In specific aspects, provided herein is an antibody or antigen-binding fragment which competitively inhibits (*e.g.*, in a dose dependent manner) binding to B7-H4 (*e.g.*, human B7-H4), of an antibody comprising a VH domain having the amino acid sequence set forth in SEQ ID NO:7, and a VL domain having the amino acid sequence set for the in SEQ ID NO:10.

[0137] In specific aspects, provided herein is an antibody or antigen-binding fragment which competitively inhibits (*e.g.*, in a dose dependent manner) binding to B7-H4 (*e.g.*, human B7-H4), of an antibody comprising a VH domain having the amino acid sequence

set forth in SEQ ID NO:8, and a VL domain having the amino acid sequence set for the in SEQ ID NO:10.

[0138] In specific aspects, provided herein is an antibody or antigen-binding fragment which competitively inhibits (*e.g.*, in a dose dependent manner) binding to B7-H4 (*e.g.*, human B7-H4), of an antibody comprising a VH domain having the amino acid sequence set forth in SEQ ID NO:6, and a VL domain having the amino acid sequence set for the in SEQ ID NO:11.

[0139] In specific aspects, provided herein is an antibody or antigen-binding fragment which competitively inhibits (*e.g.*, in a dose dependent manner) binding to B7-H4 (*e.g.*, human B7-H4), of an antibody comprising a VH domain having the amino acid sequence set forth in SEQ ID NO:7, and a VL domain having the amino acid sequence set for the in SEQ ID NO:11.

[0140] In specific aspects, provided herein is an antibody or antigen-binding fragment which competitively inhibits (*e.g.*, in a dose dependent manner) binding to B7-H4 (*e.g.*, human B7-H4), of an antibody comprising a VH domain having the amino acid sequence set forth in SEQ ID NO:8, and a VL domain having the amino acid sequence set for the in SEQ ID NO:11.

[0141] In specific aspects, provided herein is an antibody or antigen-binding fragment thereof, which immunospecifically binds to the same B7-H4 (*e.g.*, human B7-H4) epitope as that of any of J511-J522.

[0142] In a specific aspect, an antigen-binding fragment as described herein, which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4), is selected from the group consisting of a Fab, Fab', $F(ab')_2$, and scFv, wherein the Fab, Fab', $F(ab')_2$, or scFv comprises a heavy chain variable region sequence and a light chain variable region sequence of an anti-B7-H4 antibody or antigen-binding fragment thereof as described herein. A Fab, Fab', $F(ab')_2$, or scFv can be produced by any technique known to those of skill in the art, including, but not limited to, those discussed in Section 5.3, *infra*.

[0143] An anti-B7-H4 antibody or antigen-binding fragment thereof can be fused or conjugated (*e.g.*, covalently or noncovalently linked) to a detectable label or substance. Examples of detectable labels or substances include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{121}In), and technetium (^{99}Tc); luminescent labels, such as luminol; and

fluorescent labels, such as fluorescein and rhodamine, and biotin. Such labeled antibodies or antigen-binding fragments thereof can be used to detect B7-H4 (*e.g.*, human B7-H4) protein. *See*, *e.g.*, Section 5.4.1, *infra*.

Antibody Production

[0144] Antibodies and antigen-binding fragments thereof that immunospecifically bind to B7-H4 (*e.g.*, human B7-H4) can be produced by any method known in the art for the synthesis of antibodies and antigen-binding fragments thereof, for example, by chemical synthesis or by recombinant expression techniques. The methods described herein employ, unless otherwise indicated, conventional techniques in molecular biology, microbiology, genetic analysis, recombinant DNA, organic chemistry, biochemistry, PCR, oligonucleotide synthesis and modification, nucleic acid hybridization, and related fields within the skill of the art. These techniques are described, for example, in the references cited herein and are fully explained in the literature. *See*, *e.g.*, Sambrook J *et al.*, (2001) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Ausubel FM *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons (1987 and annual updates); Current Protocols in Immunology, John Wiley & Sons (1987 and annual updates) Gait (ed.) (1984) Oligonucleotide Synthesis: A Practical Approach, IRL Press; Eckstein (ed.) (1991) Oligonucleotides and Analogues: A Practical Approach, IRL Press; Birren B *et al.*, (eds.) (1999) Genome Analysis: A Laboratory Manual, Cold Spring Harbor Laboratory Press.

[0145] In a certain aspect, provided herein is a method of making an antibody or antigen-binding fragment which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4) comprising culturing a cell or host cell described herein. In a certain aspect, provided herein is a method of making an antibody or antigen-binding fragment thereof which immunospecifically binds to B7-H4 (*e.g.*, human B7-H4) comprising expressing (*e.g.*, recombinantly expressing) the antibody or antigen-binding fragment thereof using a cell or host cell described herein (*e.g.*, a cell or a host cell comprising polynucleotides encoding an antibody or antigen-binding fragment thereof described herein). In a particular embodiment, the cell is an isolated cell. In a particular embodiment, the exogenous polynucleotides have been introduced into the cell. In a particular embodiment, the method further comprises the step of purifying the antibody or antigen-binding fragment obtained from the cell or host cell.

[0146] Methods for producing polyclonal antibodies are known in the art (see, for example, Chapter 11 in: Short Protocols in Molecular Biology, (2002) 5th Ed., Ausubel FM *et al.*, eds., John Wiley and Sons, New York).

[0147] Monoclonal antibodies or antigen-binding fragments thereof can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, yeast-based presentation technologies, or a combination thereof. For example, monoclonal antibodies or antigen-binding fragments thereof can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow E & Lane D, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling GJ *et al.*, in: Monoclonal Antibodies and T-Cell Hybridomas 563 681 (Elsevier, N.Y., 1981), or as described in Kohler G & Milstein C (1975) Nature 256: 495. Examples of yeast-based presentation methods that can be employed to select and generate the antibodies described herein include those disclosed in, for example, WO2009/036379A2; WO2010/105256; and WO2012/009568, each of which is herein incorporated by reference in its entirety.

[0148] In specific embodiments, a monoclonal antibody or antigen-binding fragment thereof may be produced using the hybridoma method first described by Kohler *et al.*, Nature, 256:495 (1975), as mentioned above. In the hybridoma method, a mouse or another appropriate host animal is immunized as above described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization, for example, the B7-H4 extra cellular domain (ECD) protein (SEQ ID NO:20). Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986).

[0149] In specific embodiments, a monoclonal antibody or antigen-binding fragment thereof is an antibody or antigen-binding fragment produced by a clonal cell (*e.g.*, hybridoma or host cell producing a recombinant antibody or antigen-binding fragment), wherein the antibody or antigen-binding fragment immunospecifically binds to B7-H4 (*e.g.*, human B7-H4) as determined, *e.g.*, by ELISA or other antigen-binding assays known in the art or in the Examples provided herein. In particular embodiments, a monoclonal antibody or antigen-binding fragment thereof can be a rodent or murine antibody or antigen-binding fragment thereof. In particular embodiments, a monoclonal

antibody or antigen-binding fragment thereof can be a chimeric or a humanized antibody or antigen-binding fragment thereof. In certain embodiments, a monoclonal antibody or antigen-binding fragment thereof can be a Fab fragment or an $F(ab')_2$ fragment. Monoclonal antibodies or antigen-binding fragments thereof described herein can, for example, be made by the hybridoma method as described in Kohler G & Milstein C (1975) Nature 256: 495 or can, *e.g.*, be isolated from phage libraries using the techniques as described herein, for example. Other methods for the preparation of clonal cell lines and of monoclonal antibodies and antigen-binding fragments thereof expressed thereby are well known in the art (see, for example, Chapter 11 in: Short Protocols in Molecular Biology, (2002) 5th Ed., Ausubel FM *et al., supra*).

[0150] Antigen-binding fragments of antibodies described herein can be generated by any technique known to those of skill in the art. For example, Fab and $F(ab')_2$ fragments described herein can be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). A Fab fragment corresponds to one of the two identical arms of a tetrameric antibody molecule and contains the complete light chain paired with the VH and CH1 domains of the heavy chain. An $F(ab')_2$ fragment contains the two antigen-binding arms of a tetrameric antibody molecule linked by disulfide bonds in the hinge region.

[0151] Further, the antibodies or antigen-binding fragments thereof described herein can also be generated using various phage display and/or yeast-based presentation methods known in the art. In phage display methods, proteins are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding VH and VL domains are amplified from animal cDNA libraries (*e.g.,* human or murine cDNA libraries of affected tissues). The DNA encoding the VH and VL domains are recombined together with a scFv linker by PCR and cloned into a phagemid vector. The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13, and the VH and VL domains are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antibody or antigen-binding fragment thereof that specifically binds to a particular antigen can be selected or identified with antigen, *e.g.,* using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies or

fragments described herein include those disclosed in Brinkman U *et al.*, (1995) J Immunol Methods 182: 41-50; Ames RS *et al.*, (1995) J Immunol Methods 184: 177-186; Kettleborough CA *et al.*, (1994) Eur J Immunol 24: 952-958; Persic L *et al.*, (1997) Gene 187: 9-18; Burton DR & Barbas CF (1994) Advan Immunol 57: 191-280; PCT Application No. PCT/GB91/001134; International Publication Nos. WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/1 1236, WO 95/15982, WO 95/20401, and WO 97/13844; and U.S. Patent Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727, 5,733,743, and 5,969,108.

[0152] An antibody or antigen-binding fragment thereof can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG_1, IgG_2, IgG_3 and IgG_4.

Polynucleotides

[0153] In certain aspects, provided herein are polynucleotides comprising a nucleotide sequence encoding an antibody or antigen-binding fragment thereof described herein or a domain thereof (*e.g.*, a variable light chain region and/or variable heavy chain region) that immunospecifically binds to a B7-H4 (*e.g.*, human B7-H4) antigen, and vectors, *e.g.*, vectors comprising such polynucleotides for recombinant expression in host cells (*e.g.*, *E. coli* and mammalian cells).

[0154] In particular aspects, provided herein are polynucleotides comprising nucleotide sequences encoding antibodies or antigen-binding fragments thereof, which immunospecifically bind to a B7-H4 polypeptide (*e.g.*, human B7-H4) and comprise an amino acid sequence as described herein, as well as antibodies or antigen-binding fragments that compete with such antibodies or antigen-binding fragments for binding to a B7-H4 polypeptide (*e.g.*, in a dose-dependent manner), or which bind to the same epitope as that of such antibodies or antigen-binding fragments.

[0155] Also provided herein is a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a sequence selected from the group consisting of SEQ ID NOs:6-8. In some embodiments, an antibody or antigen-binding fragment thereof comprising the polypeptide immunospecifically binds to B7-H4.

[0156] Also provided herein is a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a sequence selected from the group consisting of SEQ

ID NOs:9-11. In some embodiments, an antibody or antigen-binding fragment thereof comprising the polypeptide immunospecifically binds to B7-H4.

[0157] Also provided herein is a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a sequence selected from the group consisting of SEQ ID NOs:22-24 or comprising the amino acids of all of SEQ ID NOs:22-24. In some embodiments, an antibody or antigen-binding fragment thereof comprising the polypeptide immunospecifically binds to B7-H4. Also provided herein is a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a sequence selected from the group consisting of SEQ ID NOs:25-29 or comprising all of SEQ ID NOs:25, 26, and 29, all of SEQ ID NOs:25, 27, and 29, or all of SEQ ID NOs:25, 28, and 29. In some embodiments, an antibody or antigen-binding fragment thereof comprising the polypeptide immunospecifically binds to B7-H4.

[0158] Also provided herein is a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a sequence selected from the group consisting of SEQ ID NOs:16-18, 89, 90, and 91. In some embodiments, an antibody or antigen-binding fragment thereof comprising the polypeptide immunospecifically binds to B7-H4.

[0159] Also provided herein is a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a sequence selected from the group consisting of SEQ ID NOs:19, 30, and 31. In some embodiments, an antibody or antigen-binding fragment thereof comprising the polypeptide immunospecifically binds to B7-H4.

[0160] Also provided herein are polynucleotides comprising a heavy chain and/or a light chain-encoding nucleotide sequence shown in Table 8, *e.g.*, wherein an antibody or antigen-binding fragment thereof comprising the encoded heavy chain or light chain specifically binds to B7-H4.

Table 8: Heavy chain and light chain-encoding polynucleotide sequences

Antibody	Heavy/Light Chain-Encoding Polynucleotide Sequence (SEQ ID NO)
J511 HC	ATGGGCAGGCTTACTTCTTCATTCCTGCTACTGATTGTCCCTGCATATGT CCTGTCCCAGGTCACTCTGAAAGAGTCTGGCCCTGGGATATTGCAGCCC TCCCAGACCCTCAGTCTGACTTGTTCTTTCTCTGGGTTTTCACTGAGCAC TTATGGTCTGGGTGTAGGTTGGATTCGTCAGCCTTCAGGGAAGGGTCTG GACTGGCTGGCCAACATTTGGTGGAATGATGATAAATACTATAACTCA GCCCTGAAGAGCCGGCTCACAATCTCCAAGGATACCTCCAACAACCAG GTATTCCTCAAGATCTCCAGTGTGGACACTGCAGATACTGGCACATACT ACTGTGCTCAAGTTGATGGTTACTACTGGTACTTCGATGTCTGGGGCGC AGGGACCACGGTCACCGTCTCCTCAGCCAAAACGACACCACCAAGTGT

	CTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACC CTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCT GGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCT GGAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGC CCTCGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGC AGCACCAAAGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAG CCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCC AAAGCCCAAGGATGTGCTCACCATTACTCTGACTCcTAAGGTCACGTGT GTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGG TTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAG GAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGC ACCAGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTG CAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGCA GACCGAAGGCTCCACAGGTGTACACCATTCCACCTCCCAAGGAGCAGA TGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTCCC TGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAA CTACAAGAACACTCAGCCCATCATGAACACGAATGGCTCTTACTTCGTC TACAGCAAGCTCAATGTGCAGAAGAGCAACTGGGAGGCAGGAAATACT TTCACCTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAGA AGAGCCTCTCCCACTCTCCTGGTAAATGA (SEQ ID NO:93)
J512, J517, J518 HC	ATGGGCAGGCTTACTTCTTCATTCCTGCTACTGATTGTCCCTGCATATGT CCTGTCCCAGGTCACTCTGAAAGAGTCTGGCCCTGGGATATTGCAGCCC TCCCAGACCCTCAGTCTGACTTGTTCTTTCTCTGGGTTTTCACTGAGCAC TTATGGTCTGGGTGTAGGTTGGATTCGTCAGCCTTCAGGGAAGGGTCTG GACTGGCTGGCCAACATTTGGTGGAATGATGATAAATACTATAACTCA GCCCTGAAGAGCCGGCTCACAATCTCCAAGGATACCTCCAACAACCAG GTATTCCTCAAGATCTCCAGTGTGGACACTGCAGATACTGGCACATACT ACTGTGCTCAAGTTGATGGTTACTACTGGTACTTCGATGTCTGGGGCGC AGGGACCACGGTCACCGTCTCCTCAGCCAAAACGACACCACCAAGTGT CTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACC CTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCT GGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCT GGAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGC CCTCGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGC AGCACCAAAGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAG CCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCC AAAGATCAAGGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGT GTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCTGG TTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGA GAGGATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGC ACCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACA AAGACCTCCCAGCGCCCATCGAGAGAACCATCTCAAAACCCAAAGGGT CAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAGA TGACTAAGAAACAGGTCACTCTGACCTGCATGGTCACAGACTTCATGCC TGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAA ACTACAAGAACACTGAACCAGTCCTGGACTCTGATGGTTCTTACTTCAT GTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAATA GCTACTCCTGTTCAGTGGTCCACGAGGGTCTGCACAATCACCACACGAC

	TAAGAGCTTCTCCCGGACTCCGGGTAAATAGTAA (SEQ ID NO:12)
J513 HC	ATGGGCAGGCTTACTTCTTCATTCCTGCTACTGATTGTCCCTGCATATGT CCTGTCCCAGGTCACTCTGAAAGAGTCTGGCCCTGGGATATTGCAGCCC TCCCAGACCCTCAGTCTGACTTGTTCTCTCTCTGGGTTTTCACTGAGCAC TTATGGTCTGGGTGTAGGTTGGATTCGTCAGCCTTCAGGGAAGGGTCTG GGCTGGCTGGCCAACATTTGGTGGAATGATGATAAATACTATAACTCA GCCCTGAAGAGCCGGCTCACAATCTCCAAGGATACCTCCAACAACCAG GTATTCCTCAAGATCTCCAGTGTGGACACTGCAGATACTGGCACATACT ACTGTGCTCAAGTTGATGGTTACTACTGGTACTTCGATGTCTGGGGCGC AGGGACCACGGTCACCGTCTCCTCAGCCAAAACGACACCACCAAGTGT CTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACC CTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCT GGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCT GGAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGC CCTCGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGC AGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAG CCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCC AAAGCCCAAGGATGTGCTCACCATTACTCTGACTCcTAAGGTCACGTGT GTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGG TTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAG GAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGC ACCAGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTG CAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGCA GACCGAAGGCTCCACAGGTGTACACCATTCCACCTCCCAAGGAGCAGA TGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTCCC TGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAA CTACAAGAACACTCAGCCCATCATGAACACGAATGGCTCTTACTTCGTC TACAGCAAGCTCAATGTGCAGAAGAGCAACTGGGAGGCAGGAAATACT TTCACCTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAGA AGAGCCTCTCCCACTCTCCTGGTAAATGA (SEQ ID NO:94)
J514, J519, J520 HC	ATGGGCAGGCTTACTTCTTCATTCCTGCTACTGATTGTCCCTGCATATGT CCTGTCCCAGGTCACTCTGAAAGAGTCTGGCCCTGGGATATTGCAGCCC TCCCAGACCCTCAGTCTGACTTGTTCTCTCTCTGGGTTTTCACTGAGCAC TTATGGTCTGGGTGTAGGTTGGATTCGTCAGCCTTCAGGGAAGGGTCTG GGCTGGCTGGCCAACATTTGGTGGAATGATGATAAATACTATAACTCA GCCCTGAAGAGCCGGCTCACAATCTCCAAGGATACCTCCAACAACCAG GTATTCCTCAAGATCTCCAGTGTGGACACTGCAGATACTGGCACATACT ACTGTGCTCAAGTTGATGGTTACTACTGGTACTTCGATGTCTGGGGCGC AGGGACCACGGTCACCGTCTCCTCAGCCAAAACGACACCACCAAGTGT CTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACC CTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCT GGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCT GGAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGC CCTCGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGC AGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAG CCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCC AAAGATCAAGGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGT GTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCTGG

	TTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGA GAGGATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGC ACCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACA AAGACCTCCCAGCGCCCATCGAGAGAACCATCTCAAAACCCAAAGGGT CAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAGA TGACTAAGAAACAGGTCACTCTGACCTGCATGGTCACAGACTTCATGCC TGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAA ACTACAAGAACACTGAACCAGTCCTGGACTCTGATGGTTCTTACTTCAT GTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAATA GCTACTCCTGTTCAGTGGTCCACGAGGGTCTGCACAATCACCACACGAC TAAGAGCTTCTCCCGGACTCCGGGTAAATAGTAA (SEQ ID NO:13)
J515 HC	ATGGGCAGGCTTACTTCTTCATTCCTGCTACTGATTGTCCCTGCATATGT CCTGTCCCAGGTCACTCTGAAAGAGTCTGGCCCTGGGATATTGCAGTCC TCCCAGACCCTCAGTCTGACTTGTTCTTTCTCTGGGTTTTCACTGAGCAC TTATGGTCTGGGTGTAGGTTGGATTCGTCAGCCTTCAGGGAAGGGTCTG GACTGGCTGGCCAACATTTGGTGGAATGATGATAAATACTATAACTCA GCCCTGAAGAGCCGGCTCACAATCTCCAAGGATACCTCCAACAACCAG GTATTCCTCAAGATCTCCAGTGTGGACACTGCAGATACTGGCACATACT ACTGTGCTCAAGTTGATGGTTACTACTGGTACTTCGATGTCTGGGGCGC AGGGACCACGGTCACCGTCTCCTCAGCCAAAACGACACCACCAAGTGT CTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACC CTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCT GGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCT GGAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGC CCTCGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGC AGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAG CCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCC AAAGCCCAAGGATGTGCTCACCATTACTCTGACTCcTAAGGTCACGTGT GTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGG TTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAG GAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGC ACCAGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTG CAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGCA GACCGAAGGCTCCACAGGTGTACACCATTCCACCTCCCAAGGAGCAGA TGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTCCC TGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAA CTACAAGAACACTCAGCCCATCATGAACACGAATGGCTCTTACTTCGTC TACAGCAAGCTCAATGTGCAGAAGAGCAACTGGGAGGCAGGAAATACT TTCACCTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAGA AGAGCCTCTCCCACTCTCCTGGTAAATGA (SEQ ID NO:95)
J516, J521, J522 HC	ATGGGCAGGCTTACTTCTTCATTCCTGCTACTGATTGTCCCTGCATATGT CCTGTCCCAGGTCACTCTGAAAGAGTCTGGCCCTGGGATATTGCAGTCC TCCCAGACCCTCAGTCTGACTTGTTCTTTCTCTGGGTTTTCACTGAGCAC TTATGGTCTGGGTGTAGGTTGGATTCGTCAGCCTTCAGGGAAGGGTCTG GACTGGCTGGCCAACATTTGGTGGAATGATGATAAATACTATAACTCA GCCCTGAAGAGCCGGCTCACAATCTCCAAGGATACCTCCAACAACCAG GTATTCCTCAAGATCTCCAGTGTGGACACTGCAGATACTGGCACATACT ACTGTGCTCAAGTTGATGGTTACTACTGGTACTTCGATGTCTGGGGCGC

	AGGGACCACGGTCACCGTCTCCTCAGCCAAAACGACACCACCAAGTGT CTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACC CTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCT GGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCT GGAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGC CCTCGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGC AGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAG CCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCC AAAGATCAAGGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGT GTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCTGG TTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGA GAGGATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGC ACCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACA AAGACCTCCCAGCGCCCATCGAGAGAACCATCTCAAAACCCAAAGGGT CAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAGA TGACTAAGAAACAGGTCACTCTGACCTGCATGGTCACAGACTTCATGCC TGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAA ACTACAAGAACACTGAACCAGTCCTGGACTCTGATGGTTCTTACTTCAT GTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAATA GCTACTCCTGTTCAGTGGTCCACGAGGGTCTGCACAATCACCACACGAC TAAGAGCTTCTCCCGGACTCCGGGTAAATAGTAA (SEQ ID NO:14)
J511 to J516 LC	ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCTTC CGGCAGTGATGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGT CTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTGTAC ATAGTAATAGAAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCC AGTCTCCAAAGCTCCTGATCTACAACGTTTCCAACCGATTTTCTGGGGT CCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAA GATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTACTGCTTTCA AGGTTCACATGTTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTG AAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTG AGCAGTTGACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTT CTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACG ACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAG CACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGACGAGTATGA ACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTC ACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG (SEQ ID NO:15)
J517, J519, and J521 LC	ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCTTC CGGCAGTGATGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGT CTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTGTAC ATAGTAATAGAAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCC AGTCTCCAAAGCTCCTGATCTACAACGTTGCCAACCGATTTTCTGGGGT CCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAA GATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTACTGCTTTCA AGGTTCACATGTTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTG AAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTG AGCAGTTGACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTT CTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACG ACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAG

	CACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGACGAGTATGA ACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTC ACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG (SEQ ID NO:96)
J518, J520, and J522 LC	ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCTTC CGGCAGTGATGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGT CTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTGTAC ATAGTAATAGAAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCC AGTCTCCAAAGCTCCTGATCTACCAGGTTTCCAACCGATTTTCTGGGGT CCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAA GATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTACTGCTTTCA AGGTTCACATGTTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTG AAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTG AGCAGTTGACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTT CTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACG ACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAG CACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGACGAGTATGA ACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTC ACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG (SEQ ID NO:97)

[0161] Also provided herein is a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:12. Also provided herein is a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:13. Also provided herein is a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:14. Also provided herein is a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:93. Also provided herein is a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:94. Also provided herein is a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:95.

[0162] Also provided herein is a polynucleotide comprising a nucleic acid encoding a light chain variable domain, wherein the nucleic acid encoding the light chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:15. Also provided

herein is a polynucleotide comprising a nucleic acid encoding a light chain variable domain, wherein the nucleic acid encoding the light chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:96. Also provided herein is a polynucleotide comprising a nucleic acid encoding a light chain variable domain, wherein the nucleic acid encoding the light chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:97.

[0163] Also provided herein is a composition comprising (i) a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:12 or 93 and (ii) a polynucleotide comprising a nucleic acid encoding a light chain variable domain, wherein the nucleic acid encoding the light chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:15, 96, or 97. The nucleic acid encoding the heavy chain variable domain and the nucleic acid encoding the light chain variable domain may be in the same polynucleotide or in different polynucleotides. The nucleic acid encoding the heavy chain variable domain and the nucleic acid encoding the light chain variable domain may be in the same vector or in different vectors.

[0164] Also provided herein is a composition comprising (i) a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:13 or 94 and (ii) a polynucleotide comprising a nucleic acid encoding a light chain variable domain, wherein the nucleic acid encoding the light chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:15, 96, or 97. The nucleic acid encoding the heavy chain variable domain and the nucleic acid encoding the light chain variable domain may be in the same polynucleotide or in different polynucleotides. The nucleic acid encoding the heavy chain variable domain and the nucleic acid encoding the light chain variable domain may be in the same vector or in different vectors.

[0165] Also provided herein is a composition comprising (i) a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:14 or 95 and (ii) a polynucleotide comprising a nucleic acid encoding a light chain variable domain, wherein the nucleic acid encoding the light chain variable domain comprises the three CDR-encoding sequence of SEQ ID NO:15, 96, or 97. The nucleic

acid encoding the heavy chain variable domain and the nucleic acid encoding the light chain variable domain may be in the same polynucleotide or in different polynucleotides. The nucleic acid encoding the heavy chain variable domain and the nucleic acid encoding the light chain variable domain may be in the same vector or in different vectors.

[0166] Also provided herein is a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:12. Also provided herein is a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:13. Also provided herein is a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:14. Also provided herein is a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:93. Also provided herein is a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:94. Also provided herein is a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:95.

[0167] Also provided herein is a polynucleotide comprising a nucleic acid encoding a light chain variable domain, wherein the nucleic acid encoding the light chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:15. Also provided herein is a polynucleotide comprising a nucleic acid encoding a light chain variable domain, wherein the nucleic acid encoding the light chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:96. Also provided herein is a polynucleotide comprising a nucleic acid encoding a light chain variable domain, wherein the nucleic acid encoding the light chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:97.

[0168] Also provided herein is a composition comprising (i) a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:12 or 93 and (ii) a polynucleotide comprising a nucleic acid encoding a light chain variable domain, wherein the nucleic acid encoding the light chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:15, 96, or 97. The nucleic acid encoding the heavy chain variable domain and the nucleic acid encoding the light chain variable domain may be in the same polynucleotide or in different polynucleotides. The nucleic acid encoding the heavy chain variable domain and the nucleic acid encoding the light chain variable domain may be in the same vector or in different vectors.

[0169] Also provided herein is a composition comprising (i) a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:13 or 94 and (ii) a polynucleotide comprising a nucleic acid encoding a light chain variable domain, wherein the nucleic acid encoding the light chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:15, 96, or 97. The nucleic acid encoding the heavy chain variable domain and the nucleic acid encoding the light chain variable domain may be in the same polynucleotide or in different polynucleotides. The nucleic acid encoding the heavy chain variable domain and the nucleic acid encoding the light chain variable domain may be in the same vector or in different vectors.

[0170] Also provided herein is a composition comprising (i) a polynucleotide comprising a nucleic acid encoding a heavy chain variable domain, wherein the nucleic acid encoding the heavy chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:14 or 95 and (ii) a polynucleotide comprising a nucleic acid encoding a light chain variable domain, wherein the nucleic acid encoding the light chain variable domain comprises the variable domain-encoding sequence of SEQ ID NO:15, 96, or 97. The nucleic acid encoding the heavy chain variable domain and the nucleic acid encoding the light chain variable domain may be in the same polynucleotide or in different polynucleotides. The nucleic acid encoding the heavy chain variable domain and the

nucleic acid encoding the light chain variable domain may be in the same vector or in different vectors.

[0171] Also provided herein are polynucleotides comprising a nucleotide sequence that encodes SEQ ID NO:6, 7, 8, 9, 10, 11, 22, 23, 24, 25, 26, 27, 28, or 29, respectively.

[0172] Also provided herein are polynucleotides that are at least about 80%, 85%, or 90% identical to a nucleotide sequence that encodes SEQ ID NO:6, 7, 8, 9, 10, 11, 22, 23, 24, 25, 26, 27, 28, or 29, respectively.

[0173] Also provided herein are polynucleotides comprising a nucleotide sequence that is at least about 95% identical to a nucleotide sequence that encodes SEQ ID NO:6, 7, 8, 9, 10, 11, 22, 23, 24, 25, 26, 27, 28, or 29, respectively. Also provided herein are polynucleotides comprising a nucleotide sequence that is at least about 96% identical to a nucleotide sequence that encodes SEQ ID NO:6, 7, 8, 9, 10, 11, 22, 23, 24, 25, 26, 27, 28, or 29, respectively. Also provided herein are polynucleotides comprising a nucleotide sequence that is at least about 97% identical to a nucleotide sequence that encodes SEQ ID NO:6, 7, 8, 9, 10, 11, 22, 23, 24, 25, 26, 27, 28, or 29, respectively. Also provided herein are polynucleotides comprising a nucleotide sequence that is at least about 98% identical to a nucleotide sequence that encodes SEQ ID NO:6, 7, 8, 9, 10, 11, 22, 23, 24, 25, 26, 27, 28, or 29, respectively. Also provided herein are polynucleotides comprising a nucleotide sequence that is at least about 99% identical to a nucleotide sequence that encodes SEQ ID NO:6, 7, 8, 9, 10, 11, 22, 23, 24, 25, 26, 27, 28, or 29, respectively.

[0174] In a particular embodiment, a polynucleotide or combination of polynucleotides provided herein comprises a nucleotide sequence or combination of nucleotide sequences encoding an antibody or antigen-binding fragment thereof that immunospecifically binds to B7-H4 (*e.g.*, human B7-H4), wherein the antibody or antigen-binding fragment thereof comprises a heavy chain, wherein the heavy chain comprises a heavy chain variable domain comprising an amino acid sequence set forth in Table 3 (*e.g.*, SEQ ID NOs:6-8) and a constant region comprising the amino acid sequence of a murine gamma (γ) heavy chain constant region (*e.g.*, IgG1 or IgG2a).

[0175] In a particular embodiment, a polynucleotide or combination of polynucleotides provided herein comprises a nucleotide sequence or combination of nucleotide sequences encoding an antibody or antigen-binding fragment thereof that immunospecifically binds to B7-H4 (*e.g.*, human B7-H4), wherein the antibody or antigen-binding fragment thereof

comprises a light chain, wherein the light chain comprises a light chain variable domain comprising an amino acid sequence set forth in Table 4 (*e.g.*, SEQ ID NOs:9-11) and a constant region comprising the amino acid sequence of a murine kappa light chain constant region.

[0176] In a specific embodiment, provided herein are polynucleotides comprising a nucleotide sequence encoding an anti-B7-H4 antibody or antigen-binding fragment thereof or a domain thereof, designated herein, *see*, *e.g.*, Table 1.

[0177] Also provided herein are polynucleotides encoding an anti-B7-H4 antibody or antigen-binding fragment thereof described herein or a domain thereof that are optimized, *e.g.*, by codon/RNA optimization, replacement with heterologous signal sequences, and elimination of mRNA instability elements. Methods to generate optimized nucleic acids encoding an anti-B7-H4 antibody or antigen-binding fragment thereof or a domain thereof (*e.g.*, heavy chain, light chain, VH domain, or VL domain) for recombinant expression by introducing codon changes (*e.g.*, a codon change that encodes the same amino acid due to the degeneracy of the genetic code) and/or eliminating inhibitory regions in the mRNA can be carried out by adapting the optimization methods described in, *e.g.*, U.S. Patent Nos. 5,965,726; 6,174,666; 6,291,664; 6,414,132; and 6,794,498, accordingly.

[0178] A polynucleotide encoding an antibody or antigen-binding fragment thereof described herein or a domain thereof can be generated from nucleic acid from a suitable source (*e.g.*, a hybridoma) using methods well known in the art (*e.g.*, PCR and other molecular cloning methods). For example, PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of a known sequence can be performed using genomic DNA obtained from hybridoma cells producing the antibody of interest. Such PCR amplification methods can be used to obtain nucleic acids comprising the sequence encoding the light chain and/or heavy chain of an antibody or antigen-binding fragment thereof. Such PCR amplification methods can be used to obtain nucleic acids comprising the sequence encoding the variable light chain region and/or the variable heavy chain region of an antibody or antigen-binding fragment thereof. The amplified nucleic acids can be cloned into vectors for expression in host cells and for further cloning, for example, to generate chimeric and humanized antibodies or antigen-binding fragments thereof.

[0179] Polynucleotides provided herein can be, *e.g.*, in the form of RNA or in the form of DNA. DNA includes cDNA, genomic DNA, and synthetic DNA, and DNA can be double-stranded or single-stranded. If single stranded, DNA can be the coding strand or non-coding (anti-sense) strand. In certain embodiments, the polynucleotide is a cDNA or a DNA lacking one more endogenous introns. In certain embodiments, a polynucleotide is a non-naturally occurring polynucleotide. In certain embodiments, a polynucleotide is recombinantly produced. In certain embodiments, the polynucleotides are isolated. In certain embodiments, the polynucleotides are substantially pure. In certain embodiments, a polynucleotide is purified from natural components.

Cells and Vectors

[0180] In certain aspects, provided herein are vectors (*e.g.*, expression vectors) comprising polynucleotides comprising nucleotide sequences encoding anti-B7-H4 antibodies and antigen-binding fragments thereof or a domain thereof for recombinant expression in host cells, preferably in mammalian cells. Also provided herein are cells, *e.g.* host cells, comprising such vectors for recombinantly expressing anti-B7-H4 antibodies or antigen-binding fragments thereof described herein. In a particular aspect, provided herein are methods for producing an antibody or antigen-binding fragments thereof described herein, comprising expressing such antibody or antigen-binding fragment thereof in a host cell.

[0181] In certain embodiments, recombinant expression of an antibody or antigen-binding fragment thereof or domain thereof described herein (*e.g.*, a heavy or light chain described herein) that specifically binds to B7-H4 (*e.g.*, human B7-H4) involves construction of an expression vector containing a polynucleotide that encodes the antibody or antigen-binding fragment thereof or domain thereof. Once a polynucleotide encoding an antibody or antigen-binding fragment thereof or domain thereof (*e.g.*, heavy or light chain variable domain) described herein has been obtained, the vector for the production of the antibody or antigen-binding fragment thereof can be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody or antigen-binding fragment thereof or domain thereof (*e.g.*, light chain or heavy chain) encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody or antigen-

binding fragment thereof or domain thereof (*e.g.*, light chain or heavy chain) coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Also provided are replicable vectors comprising a nucleotide sequence encoding an antibody or antigen-binding fragment thereof described herein, a heavy or light chain, a heavy or light chain variable domain, or a heavy or light chain CDR, operably linked to a promoter. Such vectors can, for example, include the nucleotide sequence encoding the constant region of the antibody or antigen-binding fragment thereof (see, *e.g.,* International Publication Nos. WO 86/05807 and WO 89/01036; and U.S. Patent No. 5,122,464), and variable domains of the antibody or antigen-binding fragment thereof can be cloned into such a vector for expression of the entire heavy, the entire light chain, or both the entire heavy and light chains.

[0182] An expression vector can be transferred to a cell (*e.g.*, host cell) by conventional techniques, and the resulting cells can then be cultured by conventional techniques to produce an antibody or antigen-binding fragment thereof described herein (*e.g.*, an antibody or antigen-binding fragment thereof comprising the six CDRs of SEQ ID NOs:22-25, 26, 27, or 28, and 29, the VH of SEQ ID NOs:6-8, the VL of SEQ ID NOs:9-11, the VH of SEQ ID NOs:6-8 and the VL of SEQ ID NOs:9-11, the heavy chain of SEQ ID NO:16-18, 89, 90, or 91, the light chain of SEQ ID NO:19, 30, 31, or the heavy chain of SEQ ID NOs16-18, 89, 90, or 91 and the light chain of SEQ ID NO:19, 30, or 31). Thus, provided herein are host cells containing a polynucleotide encoding an antibody or antigen-binding fragment thereof described herein *e.g.*, an antibody or antigen-binding fragment thereof comprising the six CDRs of SEQ ID NOs:22-25, 26, 27, or 28, and 29, the VH of SEQ ID NOs:6-8, the VL of SEQ ID NOs:9-11, the VH of SEQ ID NOs:6-8 and the VL of SEQ ID NOs:9-11, the heavy chain of SEQ ID NO:16-18, 89, 90, or 91, the light chain of SEQ ID NO:19, 30, or 31, or the heavy chain of SEQ ID NO:16-18, 89, 90, or 91 and the light chain of SEQ ID NO:19, 30, or 31), operably linked to a promoter for expression of such sequences in the host cell. In certain embodiments, for the expression of double-chained antibodies or antigen-binding fragments thereof, vectors encoding both the heavy and light chains, individually, can be co-expressed in the host cell for expression of the entire immunoglobulin, as detailed below. In certain embodiments, a host cell contains a vector comprising a polynucleotide encoding both the

heavy chain and light chain of an antibody described herein or a domain thereof. In specific embodiments, a host cell contains two different vectors, a first vector comprising a polynucleotide encoding a heavy chain or a heavy chain variable region of an antibody or antigen-binding fragment thereof described herein, and a second vector comprising a polynucleotide encoding a light chain or a light chain variable region of an antibody described herein (*e.g.*, an antibody comprising the six CDRs of SEQ ID NOs:22-25, 26, 27, or 28, and 29), or a domain thereof. In other embodiments, a first host cell comprises a first vector comprising a polynucleotide encoding a heavy chain or a heavy chain variable region of an antibody or antigen-binding fragment thereof described herein , and a second host cell comprises a second vector comprising a polynucleotide encoding a light chain or a light chain variable region of an antibody or antigen-binding fragment thereof described herein (*e.g.*, an antibody or antigen-binding fragment thereof comprising the six CDRs of SEQ ID NOs:22-25, 26, 27, or 28, and 29). In specific embodiments, a heavy chain/heavy chain variable region expressed by a first cell associated with a light chain/light chain variable region of a second cell to form an anti-B7-H4 antibody or antigen-binding fragment thereof described herein (*e.g.*, antibody or antigen-binding fragment thereof comprising the six CDRs of SEQ ID NOs:22-25, 26, 27, or 28, and 29). In certain embodiments, provided herein is a population of host cells comprising such first host cell and such second host cell.

[0183] In a particular embodiment, provided herein is a population of vectors comprising a first vector comprising a polynucleotide encoding a light chain/light chain variable region of an anti-B7-H4 antibody or antigen-binding fragment thereof described herein, and a second vector comprising a polynucleotide encoding a heavy chain/heavy chain variable region of an anti-B7-H4 antibody or antigen-binding fragment thereof described herein (*e.g.*, antibody or antigen-binding fragment thereof comprising the CDRs of SEQ ID NOs:22-25, 26, 27, or 28, and 29). Alternatively, a single vector can be used which encodes, and is capable of expressing, both heavy and light chain polypeptides.

[0184] A variety of host-expression vector systems can be utilized to express antibodies and antigen-binding fragments thereof described herein (*e.g.*, an antibody or antigen-binding fragment thereof comprising the CDRs of SEQ ID NOs:22-25, 26, 27, or 28, and 29) (see, *e.g.*, U.S. Patent No. 5,807,715). Such host-expression systems represent vehicles by which the coding sequences of interest can be produced and subsequently

purified, but also represent cells which can, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody or antigen-binding fragment thereof described herein *in situ*. These include but are not limited to microorganisms such as bacteria (*e.g., E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (*e.g., Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.,* baculovirus) containing antibody coding sequences; plant cell systems (*e.g.,* green algae such as *Chlamydomonas reinhardtii*) infected with recombinant virus expression vectors (*e.g.,* cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.,* Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.,* COS (*e.g.*, COS1 or COS), CHO, BHK, MDCK, HEK 293, NS0, PER.C6, VERO, CRL7O3O, HsS78Bst, HeLa, and NIH 3T3, HEK-293T, HepG2, SP210, R1.1, B-W, L-M, BSC1, BSC40, YB/20 and BMT10 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.,* metallothionein promoter) or from mammalian viruses (*e.g.,* the adenovirus late promoter; the vaccinia virus 7.5K promoter). In a specific embodiment, cells for expressing antibodies and antigen-binding fragments thereof described herein (*e.g.*, an antibody or antigen-binding fragment thereof comprising the CDRs of SEQ ID NOs:22-25, 26, 27, or 28, and 29) are CHO cells, for example CHO cells from the CHO GS System™ (Lonza). In a particular embodiment, cells for expressing antibodies described herein are human cells, *e.g.*, human cell lines. In a specific embodiment, a mammalian expression vector is pOptiVEC™ or pcDNA3.3. In a particular embodiment, bacterial cells such as *Escherichia coli*, or eukaryotic cells (*e.g.*, mammalian cells), especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary (CHO) cells in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking MK & Hofstetter H (1986) Gene 45: 101-105; and Cockett MI *et al.,* (1990) Biotechnology 8: 662-667). In certain

embodiments, antibodies or antigen-binding fragments thereof described herein are produced by CHO cells or NS0 cells.

[0185] In some embodiments, a signal peptide is used in constructing a vector containing the VH or VL of an antibody or antigen-binding fragment thereof provided herein. In one specific embodiment, the nucleotide sequence and the amino acid sequence of a signal peptide used to construct an expressing vector for the VH sequence are provided in SEQ ID NO:2 and 4, respectively. In another specific embodiment, the nucleotide sequence and the amino acid sequence of a signal peptide used to construct an expressing vector for the VL sequence are provided in SEQ ID NO:3 and 5, respectively.

[0186] In one specific embodiment, the nucleotide sequence of the VH signal peptide is provided as ATGGGC AGGCTT ACTTCT TCATTC CTGCTA CTGATT GTCCCT GCATAT GTCCTG TCC (SEQ ID NO:2). In another specific embodiment, the amino acid sequence of the VH signal peptide is provided as MGRLTSSFLLLIVPAYVLS (SEQ ID NO:4).

[0187] In one specific embodiment, the nucleotide sequence of the VL signal peptide is provided as ATGAAG TTGCCT GTTAGG CTGTTG GTGCTG ATGTTC TGGATT CCTGCT TCCGGC AGT (SEQ ID NO:3). In another specific embodiment, the amino acid sequence of the VL signal peptide is provided as MKLPVRLLVLMFWIPASGS (SEQ ID NO:5).

[0188] In addition, a host cell strain can be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products can contribute to the function of the protein. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, MDCK, HEK 293, NIH 3T3, W138, BT483, Hs578T, HTB2, BT2O and T47D, NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL7O3O, COS (*e.g.*, COS1 or COS), PER.C6, VERO, HsS78Bst, HEK-293T, HepG2, SP210, R1.1, B-W, L-M, BSC1, BSC40, YB/20, BMT10 and HsS78Bst cells. In certain embodiments, anti-B7-H4 antibodies described herein (*e.g.*, an antibody or antigen-binding fragment

thereof comprising the CDRs of SEQ ID NOs:22-25, 26, 27, or 28, and 29) are produced in mammalian cells, such as CHO cells.

[0189] In certain embodiments, anti-B7-H4 antibodies described herein (*e.g.*, an antibody or antigen-binding fragment thereof comprising the CDRs of SEQ ID NOs:22-25, 26, 27, or 28, and 29) are produced in Potelligent® CHOK1SV cells.

[0190] Once an antibody or antigen-binding fragment thereof described herein has been produced by recombinant expression, it can be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the antibodies or antigen-binding fragments thereof described herein can be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

[0191] In specific embodiments, an antibody or antigen-binding fragment thereof described herein is isolated or purified. Generally, an isolated antibody or antigen-binding fragment thereof is one that is substantially free of other antibodies or antigen-binding fragments thereof with different antigenic specificities than the isolated antibody or antigen-binding fragment thereof. For example, in a particular embodiment, a preparation of an antibody or antigen-binding fragment thereof described herein is substantially free of cellular material and/or chemical precursors.

Uses and Methods

Detection & Diagnostic Uses

[0192] An anti-B7-H4 antibody or antigen-binding fragment thereof described herein (see, *e.g.*, Section 5.2) can be used to assay B7-H4 protein levels in a biological sample using methods known to those of skill in the art, including immunoassays, such as the enzyme linked immunosorbent assay (ELISA), fluorescence-activated cell sorting (FACS), immunohistochemistry (IHC), immunoprecipitation, and Western blotting.

[0193] Suitable antibody assay labels are known in the art and include enzyme labels, such as, horseradish peroxidase (HRP) and glucose oxidase; radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{121}In), and technetium (^{99}Tc);

haptens, fluorescent labels, phosphorescent molecules, chemiluminescent molecules, chromophores, luminescent molecules, photoaffinity molecules, colored particles and/or ligands, such as biotin. In some embodiments, an enzyme (an enzyme tag) will generate a colored product upon contact with a chromogenic substrate. Examples of suitable enzymes include urease, alkaline phosphatase, (horseradish) hydrogen peroxidase and/or glucose oxidase.

[0194] Such labels can be used to label an antibody or antigen-binding fragment thereof described herein. Alternatively, a second antibody or antigen-binding fragment thereof that recognizes an anti-B7-H4 antibody or antigen-binding fragment thereof described herein can be labeled and used in combination with an anti-B7-H4 antibody or antigen-binding fragment thereof to detect B7-H4 protein levels. In some embodiments, such a secondary antibody or antigen-binding fragment thereof, *e.g.*, an anti-mouse or anti-rodent antibody, is labeled with an enzyme (*e.g.*, horseradish peroxidase) and detected with a substrate of the enzyme (*e.g.*, 3,3'-diaminobenzidine (DAB)).

[0195] Several methods are known in the art for the attachment or conjugation of an antibody to its conjugate moiety. Some attachment methods involve the use of a metal chelate complex employing, for example, an organic chelating agent such a diethylenetriaminepentaacetic acid anhydride (DTPA); ethylenetriaminetetraacetic acid; N-chloro-p-toluenesulfonamide; and/or tetrachloro-3α-6α-diphenylglycouril-3 attached to the antibody (U.S. Pat. Nos. 4,472,509 and 4,938,948, each incorporated herein by reference). Monoclonal antibodies may also be reacted with an enzyme in the presence of a coupling agent such as glutaraldehyde or periodate. Conjugates with fluorescein markers are prepared in the presence of these coupling agents or by reaction with an isothiocyanate. In U.S. Pat. No. 4,938,948, imaging of breast tumors, for example, is achieved using monoclonal antibodies, and the detectable imaging moieties are bound to the antibody using linkers such as methyl-p-hydroxybenzimidate or N-succinimidyl-3-(4-hydroxyphenyl)propionate.

[0196] In other embodiments, derivatization of immunoglobulins by selectively introducing sulfhydryl groups in the Fc region of an immunoglobulin using reaction conditions that do not alter the antibody combining site are contemplated. Antibody conjugates produced according to this methodology are disclosed to exhibit improved longevity, specificity and sensitivity (U.S. Pat. No. 5,196,066, incorporated herein by

reference). Site-specific attachment of effector or reporter molecules, wherein the reporter or effector molecule is conjugated to a carbohydrate residue in the Fc region, has also been disclosed in the literature (O'Shannessy *et al.*, Biotechnol Appl Biochem. 1987 Dec;9(6):488-96).

[0197] Assaying for the expression level of B7-H4 protein is intended to include qualitatively or quantitatively measuring or estimating the level of a B7-H4 protein in a first biological sample either directly (*e.g.*, by determining or estimating absolute protein level) or relatively (*e.g.*, by comparing to B7-H4 protein level in a second biological sample or standard). B7-H4 polypeptide expression level in the first biological sample can be measured or estimated and compared to a standard B7-H4 protein level, the standard being determined from a second biological sample that is not diseased or being determined by averaging levels from a population of samples that are not diseased. As will be appreciated in the art, once the "standard" B7-H4 polypeptide level is known, it can be used repeatedly as a standard for comparison.

[0198] As used herein, the term "biological sample" refers to any biological sample obtained from a subject, cell line, tissue, or other source of cells potentially expressing B7-H4. Non-limiting sources of a biological sample for use in the present invention include solid tissue, biopsy, ascites, aspirates, fluidic extracts, blood (including circulating tumor cells), plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, tumors, organs, cell cultures and/or cell culture constituents, for example. Methods for obtaining tissue biopsies and body fluids from animals (*e.g.*, humans) are well known in the art. Exemplary methods of obtaining circulating tumor cells (CTCs) are disclosed in Ferreira *et al.*, *Molecular Oncology 10*: 374-394, which is herein incorporated by reference in its entirety. For instance, CTCs can be enriched from a blood sample using immunoaffinity-based or biophysically-based strategies. CTCs can also be obtained using direct imaging modalities. Exemplary methods of obtaining and processing biopsies, which can be used in immunohistochemical (IHC) assays are provided herein in Example 4.

[0199] An anti-B7-H4 antibody described herein can be used for diagnostic applications, including *in vitro* applications well known and standard to the skilled artisan and based on the present description. Diagnostic assays and kits for *in vitro* assessment of B7-H4 may be utilized to evaluate patient samples including those known to have or suspected of

having cancer. This type of prognostic and diagnostic monitoring and assessment is in practice, e.g., utilizing antibodies against the HER2 protein in breast cancer (HercepTest™, Dako) in which the assay is used to evaluate patients for antibody therapy using Herceptin®.

[0200] Anti-B7-H4 antibodies and antigen-binding fragments thereof described herein can carry a detectable or functional label. When fluorescence labels are used, currently available microscopy and fluorescence-activated cell sorter analysis (FACS) or combination of both methods procedures known in the art may be utilized to identify and to quantitate the specific binding members. Anti-B7-H4 antibodies or antigen-binding fragments thereof described herein can carry a fluorescence label. Exemplary fluorescence labels include, for example, reactive and conjugated probes, *e.g.*, Aminocoumarin, Fluorescein and Texas red, Alexa Fluor dyes, Cy dyes and DyLight dyes. An anti-B7-H4 antibody can carry a radioactive label, such as the isotopes ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{67}Cu, ^{90}Y, ^{99}Tc, ^{111}In, ^{117}Lu, ^{121}I, ^{124}I, ^{125}I, ^{131}I, ^{198}Au, ^{211}At, ^{213}Bi, ^{225}Ac and ^{186}Re. When radioactive labels are used, currently available counting procedures known in the art may be utilized to identify and quantitate the specific binding of anti-B7-H4 antibody or antigen-binding fragment to B7-H4 (*e.g.*, human B7-H4). In the instance where the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques as known in the art. This can be achieved by contacting a sample or a control sample with an anti-B7-H4 antibody or antigen-binding fragment thereof under conditions that allow for the formation of a complex between the antibody or antigen-binding fragment thereof and B7-H4. Any complexes formed between the antibody or antigen-binding fragment thereof and B7-H4 are detected and compared in the sample and the control. In light of the specific binding of the antibodies or antigen-binding fragments thereof described herein for B7-H4, the antibodies or antigen-binding fragments thereof can be used to specifically detect B7-H4 expression, *e.g.*, in whole cells, on cell membranes, or in cytoplasm. The antibodies or antigen-binding fragments thereof described herein can also be used to purify B7-H4 via immunoaffinity purification.

[0201] Also included herein is an assay system which may be prepared in the form of a test kit for the quantitative analysis of the extent of the presence of, for instance, B7-H4.

The system or test kit may comprise a labeled component, *e.g.*, a labeled antibody or antigen-binding fragment, and one or more additional immunochemical reagents. *See*, *e.g.*, Section 5.4.3 below for more on kits.

[0202] In some aspects, methods for detecting B7-H4 in a sample *in vitro*, comprise contacting the sample with an antibody or antigen-binding fragment thereof as provided herein. In some aspects, provided herein is the use of an antibody or antigen-binding fragment thereof as provided herein, for detecting B7-H4 in a sample *in vitro*. In one aspect, provided herein is an antibody or antigen-binding fragment thereof provided herein for use in the detection of B7-H4 in a subject or a sample obtained from a subject. In one aspect, provided herein is an antibody or antigen-binding fragment thereof provided herein for use as a diagnostic. In one embodiment, the antibody comprises a detectable label. In one preferred embodiment, B7-H4 is human B7-H4. In one preferred embodiment, the subject is a human. In a further embodiment, the subject, e.g., a human subject, has cancer.

[0203] In some aspects, the present invention contemplates immunodetection methods for binding and detecting B7-H4. The antibodies prepared in accordance with the present invention may be employed to detect B7-H4. Some immunodetection methods include immunohistochemistry, flow cytometry, enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoradiometric assay, fluoroimmunoassay, chemiluminescent assay, bioluminescent assay, and Western blot to mention a few. The steps of various useful immunodetection methods have been described in the scientific literature, such as, *e.g.*, Doolittle M H and Ben-Zeev O, Methods Mol Biol. 1999;109:215-37; Gulbis B and Galand P, Hum Pathol. 1993 Dec;24(12):1271-85; and De Jager R *et al.*, Semin Nucl Med. 1993 Apr;23(2):165-79, each incorporated herein by reference.

[0204] In general, the immunobinding methods include obtaining a sample, *e.g.* a sample suspected of comprising B7-H4, and contacting the sample with a first anti-B7-H4 antibody in accordance with the present invention under conditions effective to allow the formation of immunocomplexes.

[0205] Contacting the chosen biological sample with the antibody under effective conditions and for a period of time sufficient to allow the formation of immune complexes (primary immune complexes) generally comprises adding the antibody

composition to the sample and incubating the mixture for a period of time sufficient for the antibodies to form immune complexes with, i.e., to specifically bind to, any B7-H4 present. After this time, the sample-antibody composition, such as a tissue section, ELISA plate, dot blot or western blot, will generally be washed to remove any non-specifically bound antibody species, allowing only those antibodies specifically bound within the primary immune complexes to be detected.

[0206] In general, the detection of immunocomplex formation is well known in the art and may be achieved through the application of numerous approaches. These methods are generally based upon the detection of a label or marker, such as any of those radioactive, fluorescent, biological and enzymatic tags. U.S. Patents concerning the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241, each incorporated herein by reference. In some embodiments, a secondary binding agent, such as a second antibody and/or a biotin/avidin ligand binding arrangement, may be used in accordance with methodologies known in the art.

[0207] In some embodiments, the first antibody that becomes bound within the primary immune complexes may be detected by means of a second binding agent that has binding affinity for the antibody. In these cases, the second binding agent may be linked to a detectable label. The second binding agent is itself often an antibody, which may thus be termed a "secondary" antibody. The primary immune complexes are contacted with the labeled, secondary binding agent, or antibody, under effective conditions and for a period of time sufficient to allow the formation of secondary immune complexes. The secondary immune complexes are then generally washed to remove any non-specifically bound labeled secondary antibodies or ligands, and the remaining label in the secondary immune complexes is then detected.

[0208] Further methods include the detection of primary immune complexes by a two-step approach. A second binding agent, such as an antibody, that has binding affinity for the antibody is used to form secondary immune complexes, as described above. After washing, the secondary immune complexes are contacted with a third binding agent or antibody that has binding affinity for the second antibody, again under effective conditions and for a period of time sufficient to allow the formation of immune complexes (tertiary immune complexes). The third ligand or antibody is linked to a

detectable label, allowing detection of the tertiary immune complexes thus formed. This system may provide for signal amplification if this is desired.

[0209] In another embodiment, a biotinylated monoclonal or polyclonal antibody is used to detect the target antigen(s), and a second step antibody is then used to detect the biotin attached to the complexed antibody. In that method the sample to be tested is first incubated in a solution comprising the first step antibody. If the target antigen is present, some of the antibody specifically binds to the antigen to form a biotinylated antibody/antigen complex. The antibody/antigen complex is then amplified by incubation in successive solutions of streptavidin (or avidin) and biotinylated DNA, and/or complementary biotinylated DNA, with each step adding additional biotin sites to the antibody/antigen complex. The amplification steps are repeated until a suitable level of amplification is achieved, at which point the sample is incubated in a solution comprising the second step antibody against biotin. This second step antibody is labeled, as for example with an enzyme that can be used to detect the presence of the antibody/antigen complex by histoenzymology using a chromogen substrate. With suitable amplification, a conjugate can be produced that is macroscopically visible.

[0210] In one embodiment, immunohistochemistry (IHC) is used for immunological detection. Using IHC, detection of B7-H4 in a sample can be achieved by targeting a sample with a binding agent, *e.g.*, an anti-B7-H4 antibody or antigen-binding fragment thereof. The binding agent can be linked, either directly or indirectly to a detectable label or can be detected by another binding agent that is linked, either directly or indirectly to a detectable label. In one embodiment, 3,3'-diaminobenzidine (DAB) is used in the IHC assay to detect the primary antibody bound to B7-H4. In one embodiment, the concentration of the anti-B7-H4 antibody or antigen-binding fragment thereof in the IHC assay is about 1 μg/ml to about 50 μg/ml. In one embodiment, the concentration of the anti-B7-H4 antibody or antigen-binding fragment thereof in the IHC assay is about 1 μg/ml to about 20 μg/ml. In one embodiment, the concentration of the anti-B7-H4 antibody or antigen-binding fragment thereof in the IHC assay is about 10 μg/ml.

[0211] IHC can be performed on cells, cell pellets, tissues, preparations from blood, plasma, serum, or lymph fluid, etc. In some embodiments, the samples are fixed samples. In some embodiments, the samples are paraffin embedded samples. In some embodiments, the samples are formalin fixed and paraffin embedded samples.

[0212] In one embodiment, flow cytometry is used for immunological detection. Thus, for example, the number of antibodies bound per cell (ABC) can be assessed using flow cytometry.

Therapeutic Uses and Methods

[0213] In one aspect, presented herein are methods for treating a B7-H4-expressing cancer in a subject comprising administering a therapeutic anti-B7-H4 antibody or antigen-binding fragment thereof to the subject, wherein B7-H4 expression has been detected in a sample obtained from the subject using an antibody or antigen-binding fragment thereof provided herein. In some embodiments, the method further comprises detecting the B7-H4 in the sample obtained from the subject.

[0214] In a certain embodiment, provided herein the cancer is selected from the group consisting of: breast cancer (*e.g.*, triple negative breast cancer, hormone receptor (HR) positive breast cancer, ductal carcinoma), endometrial carcinoma, ovarian cancer, non-small cell lung cancer (*e.g.*, squamous cell carcinoma), pancreatic cancer, thyroid cancer, kidney cancer (*e.g.*, renal cell carcinoma), and bladder cancer (*e.g.*, urothelial cell carcinoma).

[0215] Therapeutic anti-B7-H4 antibodies or antigen-binding fragments thereof include, for example, 20502 and 22213 and antigen-binding fragments thereof. Amino acid and nucleotide sequences for the 20502 and 22213 antibodies are provided in Tables 9 and 10.

Table 9: 20502 Antibody Amino Acid and Nucleotide Sequences

Antibody Domain (AA/N)	**Sequence (SEQ ID NO)**
VH CDR1 (AA)	GSIKSGSYYWG (SEQ ID NO:58)
VH CDR2 (AA)	NIYYSGSTYYNPSLRS (SEQ ID NO:59)
VH CDR3 (AA)	AREGSYPNQFDP (SEQ ID NO:60)
VL CDR1 (AA)	RASQSVSSNLA (SEQ ID NO:61)
VL CDR2 (AA)	GASTRAT (SEQ ID NO:62)
VL CDR3 (AA)	QQYHSFPFT (SEQ ID NO:63)
VH (AA)	QLQLQESGPGLVKPSETLSLTCTVSGGSIKSGSYYWGWIR QPPGKGLEWIGNIYYSGSTYYNPSLRSRVTISVDTSKNQFS LKLSSVTAADTAVYYCAREGSYPNQFDPWGQGTLVTVSS (SEQ ID NO:64)
VL (AA)	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKP GQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQSED FAVYYCQQYHSFPFTFGGGTKVEIK (SEQ ID NO:65)
VH FR1 (AA)	QLQLQESGPGLVKPSETLSLTCTVSG (SEQ ID NO:66)

VH FR2 (AA)	WIRQPPGKGLEWIG (SEQ ID NO:67)
VH FR3 (AA)	RVTISVDTSKNQFSLKLSSVTAADTAVYYC (SEQ ID NO:68)
VH FR4 (AA)	WGQGTLVTVSS (SEQ ID NO:69)
VL FR1 (AA)	EIVMTQSPATLSVSPGERATLSC (SEQ ID NO:70)
VL FR2 (AA)	WYQQKPGQAPRLLIY (SEQ ID NO:71)
VL FR3 (AA)	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC (SEQ ID NO:72)
VL FR4 (AA)	FGGGTKVEIK (SEQ ID NO:73)
Heavy Chain (AA)	QLQLQESGPGLVKPSETLSLTCTVSGGSIKSGSYYWGWIR QPPGKGLEWIGNIYYSGSTYYNPSLRSRVTISVDTSKNQFS LKLSSVTAADTAVYYCAREGSYPNQFDPWGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:74)
Light Chain (AA)	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKP GQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQSED FAVYYCQQYHSFPFTFGGGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC (SEQ ID NO:75)
VH (N)	CAGCTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGA AGCCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTCTG GTGGCTCCATCAAAAGTGGTAGTTACTACTGGGGCTGG ATCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGGATTG GGAACATCTATTATAGTGGGAGCACCTACTACAACCCG TCCCTCAGAAGTCGAGTCACCATATCCGTAGACACGTC CAAGAACCAGTTCTCCCTGAAGCTGAGTTCTGTGACCG CCGCAGACACGGCGGTGTACTACTGCGCCAGAGAAGG ATCTTACCCCAATCAGTTTGATCCATGGGGACAGGGTA CATTGGTCACCGTCTCCTCA (SEQ ID NO:76)
VL(N)	GAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGT GTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCA GTCAGAGTGTTAGCAGCAACTTAGCCTGGTACCAGCAG AAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGC ATCCACCAGGGCCACTGGTATCCCAGCCAGGTTCAGTG GCAGTGGGTCTGGGACAGAGTTCACTCTCACCATCAGC AGCCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCA GCAGTACCACTCCTTCCCTTTCACTTTTGGCGGAGGGA CCAAGGTTGAGATCAAA (SEQ ID NO:77)

Table 10: 22213 Antibody Amino Acid and Nucleotide Sequences

Antibody Domain (AA/N)	Sequence (SEQ ID NO)
VH CDR1 (AA)	GSIGRGSYYWG (SEQ ID NO:32)
VH CDR2 (AA)	NIYYSGSTYYNPSLKS (SEQ ID NO:33)
VH CDR3 (AA)	AREGSYTTVLNV (SEQ ID NO:34)
VL CDR1 (AA)	RASQSVASSHLA (SEQ ID NO:35)
VL CDR2 (AA)	DAVSRAT (SEQ ID NO:36)
VL CDR3 (AA)	QQAASYPLT (SEQ ID NO:37)
VH (AA)	QLQLQESGPGLVKPSETLSLTCTVSGGSIGRGSYYWGWIR QPPGKGLEWIGNIYYSGSTYYNPSLKSRVTISVDTSKNQFS LKLSSVTAADTAVYYCAREGSYTTVLNVWGQGTMVTVS S (SEQ ID NO:54)
VL (AA)	EIVLTQSPGTLSLSPGERATLSCRASQSVASSHLAWYQQK PGQAPRLLIYDAVSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQAASYPLTFGGGTKVEIK (SEQ ID NO:55)
VH FR1 (AA)	QLQLQESGPGLVKPSETLSLTCTVSG (SEQ ID NO:78)
VH FR2 (AA)	WIRQPPGKGLEWIG (SEQ ID NO:79)
VH FR3 (AA)	RVTISVDTSKNQFSLKLSSVTAADTAVYYC (SEQ ID NO:80)
VH FR4 (AA)	WGQGTMVTVSS (SEQ ID NO:81)
VL FR1 (AA)	EIVLTQSPGTLSLSPGERATLSC (SEQ ID NO:82)
VL FR2 (AA)	WYQQKPGQAPRLLIY (SEQ ID NO:83)
VL FR3 (AA)	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC (SEQ ID NO:84)
VL FR4 (AA)	FGGGTKVEIK (SEQ ID NO:98)
Heavy Chain (AA)	QLQLQESGPGLVKPSETLSLTCTVSGGSIGRGSYYWGWIR QPPGKGLEWIGNIYYSGSTYYNPSLKSRVTISVDTSKNQFS LKLSSVTAADTAVYYCAREGSYTTVLNVWGQGTMVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:56)
Light Chain (AA)	EIVLTQSPGTLSLSPGERATLSCRASQSVASSHLAWYQQK PGQAPRLLIYDAVSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQAASYPLTFGGGTKVEIKRTVAAPSVFIFPPS DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC (SEQ ID NO:57)
VH (N)	CAGCTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGA AGCCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTCTG GTGGCTCCATCGGGAGGGGGAGTTACTACTGGGGCTGG

	ATCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGGATTG GGAACATCTATTATAGTGGGAGCACCTACTACAACCCG TCCCTCAAGAGTCGAGTCACCATATCCGTAGACACGTC CAAGAACCAGTTCTCCCTGAAGCTGAGTTCTGTGACCG CCGCAGACACGGCGGTGTACTACTGCGCCAGAGAAGG ATCTTACACAACCGTGTTAAACGTATGGGGTCAGGGTA CAATGGTCACCGTCTCCTCA (SEQ ID NO:85)
VL (N)	GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTT GTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCA GTCAGAGTGTTGCCAGCAGCCACTTAGCCTGGTACCAG CAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGA CGCAGTCAGCAGGGCCACTGGCATCCCAGACAGGTTCA GTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATC AGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTG TCAGCAGGCCGCCAGTTACCCTCTCACTTTTGGCGGAG GGACCAAGGTTGAGATCAAA (SEQ ID NO:86)

[0216] In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 of 20502. The CDRs can be the Kabat-defined CDRs, the Chothia-defined CDRs, or the AbM-defined CDRs.

[0217] In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 of 22213. The CDRs can be the Kabat-defined CDRs, the Chothia-defined CDRs, or the AbM-defined CDRs.

[0218] In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 comprise the amino acid sequences of SEQ ID NOs:32-37, respectively. In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 comprise the amino acid sequences of SEQ ID NOs:58-63, respectively.

[0219] In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:54. In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:64.

[0220] In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO:55. In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO:65.

[0221] In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:54 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:55. In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:64 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:65.

[0222] In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:56. In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:74.

[0223] In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises a light chain comprising the amino acid sequence of SEQ ID NO:57. In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises a light chain comprising the amino acid sequence of SEQ ID NO:75.

[0224] In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:56 and a light chain comprising the amino acid sequence of SEQ ID NO:57. In some embodiments, the therapeutic anti-B7-H4 antibody or antigen-binding fragment comprises) a heavy chain comprising the amino acid sequence of SEQ ID NO:74 and a light chain comprising the amino acid sequence of SEQ ID NO:75.

Kits

[0225] Provided herein are kits comprising one or more antibodies or antigen-binding fragments thereof described herein or conjugates (e.g., detection conjugates) thereof. As

provided herein, kits can be used in diagnostic methods. In one embodiment, a kit comprises an antibody or antigen-binding fragment thereof described herein, preferably a purified antibody or antigen-binding fragment thereof, in one or more containers.

[0226] In a specific embodiment, kits described herein contain a substantially isolated B7-H4 antigen (*e.g.*, human B7-H4) that can be used as a control. In specific embodiments, a kit provided herein can include a recombinantly produced or chemically synthesized B7-H4 antigen. The B7-H4 antigen provided in the kit can also be attached to a solid support.

[0227] In another specific embodiment, the kits described herein further comprise a control antibody or antigen-binding fragment thereof which does not react with a B7-H4 antigen. In another specific embodiment, kits described herein contain one or more elements for detecting the specific binding of an antibody or antigen-binding fragment thereof to a B7-H4 antigen (*e.g.*, the antibody or antigen-binding fragment thereof can be conjugated to a detectable substrate such as a fluorescent compound, an enzyme, an enzymatic substrate, a radioactive compound, or a luminescent compound, or a second antibody or antigen-binding fragment thereof, which recognizes the first antibody or antigen-binding fragment thereof, can be conjugated to a detectable substrate). In a more specific embodiment, the detecting elements of the above described kit includes a solid support to which a B7-H4 antigen is attached. Such a kit can also include a non-attached reporter-labeled anti-mouse/rodent antibody or antigen-binding fragment thereof. In this embodiment, binding of the antibody or antigen-binding fragment thereof to the B7-H4 antigen can be detected by binding of the said reporter-labeled antibody or antigen-binding fragment thereof.

[0228] In another specific embodiment, the kits described herein further comprise a therapeutic anti-B7-H4 antibody or antigen-binding fragment thereof and/or information that a therapeutic anti-B7-H4 antibody or antigen-binding fragment thereof should be administered when B7-H4 is detected in a sample using an anti-B7-H4 antibody or antigen-binding fragment thereof provided herein.

[0229] The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

[0230] The examples in this Section (i.e., Section 6) are offered by way of illustration, and not by way of limitation.

Example 1: Hybridoma Generation

[0231] A panel of antibodies that selectively bind B7-H4 were generated using a recombinant soluble version of the B7-H4 extra cellular domain (ECD) protein: MASLGQILFWSIISIIIILAGAIALIIGFGISGRHSITVTTVASAGNIGEDGILSCTFEPDIKLSDI VIQWLKEGVLGLVHEFKEGKDELSEQDEMFRGRTAVFADQVIVGNASLRLKNVQLTDA GTYKCYIITSKGKGNANLEYKTGAFSMPEVNVDYNASSETLRCEAPRWFPQPTVVWAS QVDQGANFSEVSNTSFELNSENVTMKVVSVLYNVTINNTYSCMIENDIAKATGDIKVTES EIKRRSHLQLLNSKASGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK (SEQ ID NO:20).

[0232] Each hind footpad of a NZB/W mouse was injected with B7-H4 ECD protein resuspended in Sigma Adjuvant System® (Sigma-Aldrich, Inc., St. Louis, MO). Serum was taken 21 days after first boost and titered by enzyme-linked immunosorbant assay (ELISA) and fluorescence-activated cell sorting (FACS) and flow cytometry to ensure that the mouse had a good immune response. The ELISA and FACS screenings are described in detail in Examples 2 and 3, respectively. Three days after the final boost, popliteal node cells from titer-positive mice were fused with a mouse myeloma cell line (see, for example, Chuntharapai *et al.*, 1997, Methods Enzymol. 288: 15-27). About 2880 rat hybridoma clones were generated.

[0233] Hybridoma clones generated by this process were then screened for production of monoclonal antibodies binding to the B7-H4 ECD protein (SEQ ID NO:20), the B7-H4 N-Terminal IgV-domain (amino acids 1-151)-huIgG Fc fusion protein (SEQ ID NO:21), and to HEK 293 cells stably expressing full length human B7-H4 (SEQ ID NO:1) on their surface.

MASLGQILFWSIISIIIILAGAIALIIGFGISGRHSITVTTVASAGNIGEDGILSCTFEPDIKLSDI VIQWLKEGVLGLVHEFKEGKDELSEQDEMFRGRTAVFADQVIVGNASLRLKNVQLTDA

GTYKCYIITSKGKGNANLEYKTGAFSGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:21).

Example 2: ELISA Screening

[0234] To screen the hybridoma clones generated in Example 1, ELISA was performed generally as described in Baker *et al.*, 2002, Trends Biotechnol, 20:149-156.

[0235] Briefly, 96-well plates were coated with 50 µl of the B7-H4 ECD protein (SEQ ID NO:20) or the B7-H4 N-Terminal Fc fusion protein (SEQ ID NO:21) at a concentration of 2 µg/ml in coating buffer (0.05 M carbonate buffer, pH 9.6), sealed, and stored overnight at 40°C. After removing the coating solution, 200 µl of the assay/blocking solution containing 0.5% bovine serum albumin (BSA) and 0.05% Tween®-20 in PBS (pH 7.4) (ELISA diluent) was added to each well of a 96-well plate. The plates were incubated at room temperature for one hour with agitation. The wells were then washed three times with 300 µl of 0.05% Tween®-20 in PBS (wash buffer). After washing, 100 µl of hybridoma supernatant in ELISA diluent was added to each well, and the plates were incubated at room temperature for one hour with agitation. The wells were washed three times with wash buffer as previously described. After washing, 100 µl of a 1:1000 dilution of sheep anti-mouse IgG coupled to horseradish peroxidase in ELISA diluent was added to each well. The plates were incubated at room temperature for one hour with agitation, washed three times with wash buffer as previously described, and patted dry. The wells were developed by adding 100 µl of tetramethylbenzidine (TMB) microwell peroxidase substrate to each well and incubating at room temperature for 5-10 minutes or until a good color change was observed. Development was stopped by adding 100 µl of TMB Stop Solution to each well. Plates were analyzed at 650 nm.

[0236] Prebleed and polysera were used as controls. Prebleed samples contained mouse sera prior to immunization, and polysera samples contained mouse anti-sera obtained after immunizations. Antibodies that gave a positive ELISA signal were selected for further screening by fluorescence-activated cell sorting (FACS).

Example 3: FACS Screening

[0237] To further screen the hybridoma clones generated in Example 1, fluorescence-activated cell sorting (FACS) was performed using HEK 293 cells stably expressing the full length human B7-H4 (SEQ ID NO:1). Standard HEK 293 cell lines served as negative controls. Cells were resuspended and centrifuged at 500g for 5 minutes at 40°C. Media was aspirated and cells were resuspended in BD FACSFlow™ Stain Buffer (BD Biosciences, San Jose, CA) with 1% fetal bovine serum (FBS) (cell staining buffer) at 40°C. Cells were centrifuged as previously described, media were aspirated, and cells were resuspended in cell staining buffer at 40°C to a final concentration of $2x10^6$ cells/ml. Cells were then added to 96-well round bottom plates at 50 µl/well. 100 µl of supernatant from each hybridoma clone were added to each well so that each hybridoma supernatant was incubated with one well containing either the HEK 293 cell line stably expressing B7-H4 or the negative control cell line. The plates were incubated on ice for 30 minutes and centrifuged as previously described. Then the supernatants were aspirated. Each well was resuspended in 200 µl of cell staining buffer at 40°C. Subsequently, the plates were centrifuged as previously described, and the cell staining buffer was aspirated. After the washing step, cells in each well were resuspended in 100 µl of a 1:1000 dilution of goat anti-mouse IgG Fc coupled to R-phycoerythrin (Jackson Immunoresearch, West Grove, PA) in cell staining buffer at 40°C, and the plates were incubated in the dark on ice for 30 minutes. The plates were centrifuged as previously described, and the supernatants were aspirated. Each well was resuspended in 200 µl of cell staining buffer at 40°C, and the plates were centrifuged as previously described. The cell staining buffer was aspirated. Cells in each well were resuspended in 200 µl of cell staining buffer at 40°C and transferred to 1.2 ml micro titertubes. FACS was performed on a FACScan™ or FACSCalibur™ (BD Biosciences, San Jose, CA). Antibodies that showed cell binding in the FACS assay were selected for further screening by immunohistochemistry (IHC).

Example 4: IHC Screening

[0238] To further screen the hybridoma clones generated in Example 1, immunohistochemical (IHC) staining was performed on sections obtained from a subject with an invasive ductal carcinoma breast tumor. Formalin-fixed paraffin embedded (FFPE) sections were cut at 5 micron, air dried on Superfrost Plus charged slides, and

baked for one hour at 60°C. Sections were deparaffinized and stained on a Discovery Ultra autostainer (Ventana Medical Systems, Tucson, AZ). After 1 hour of antigen retrieval with Ultra CC1 at 95°C, B7-H4 was detected with anti-B7-H4 mouse primary antibodies for 1 hour at room temperature followed by Omni-Map anti-Mouse HRP, ChromoMap 3,3'-diaminobenzidine (DAB) detection system and hematoxylin counterstaining, as per the manufacturer's instructions.

[0239] Seven anti-B7-H4 primary antibodies were used: six recombinant antibodies generated in Example 1 (at 10 μg/mL) along with A57.1 (see U.S. Patent No. 7,619,068) (at 1.25 μg/mL), which was used as a control. The two recombinant antibodies (J516 and J512) detected B7-H4 on cell membranes and cytoplasm in a similar pattern to A57.1, with a majority of B7-H4-positive cells observed in the tumor compartment, as shown in Figures 1 and 2. The recombinant antibodies detected fewer B7-H4-expressing cells with 1+ signal intensity than A57.1 (35% by J512 vs 35% by J516% vs 58% by A57.1), as shown by Figures 3 and 4. When B7-H4 expression was measured by computational image analysis (Definiens, Cambridge, MA), J516 and J512 demonstrated a similar distribution of B7-H4 signal across the cellular compartments to A57.1, as shown in Figures 5A-5C.

Example 5: Sequence Determination

[0240] The sequence of the heavy chain (HC) and light chain (LC) anti-B7-H4 antibodies M6, M11, and M15, all of which showed IHC staining, were determined according to the following procedure.

[0241] RNA was harvested from hybridomas using Trizol reagent (Thermofisher Scientific, Carlsbad, CA), and cDNA was prepared using Smartscribe reverse transcriptase (Clontech, Mountain View, CA) and Advantage 2 polymerase (Clontech, Mountain View, CA). PCR was done using Phusion Taq polymerase (NEB, Ipswich, MA) under standard PCR conditions. PCR products were then cloned using Zero Blunt ® TOPO® cloning kit (Thermofisher Scientific, Carlsbad, CA). Clones were screened for inserts, and multiple clones were sequenced to verify the original heavy chain and light chain gene sequences. The heavy chain and light chain-encoding polynucleotide sequences are provided in Table 8 above.

[0242] Anti-B7-H4 antibody variable domains were then subcloned into expression vectors with different Fc regions to generate antibodies with mouse IgG2a and mouse

IgG1 constant regions. Anti-B7-H4 heavy chain variable domains were then cloned into expression vectors with different Fc regions to generate antibodies with mouse IgG2a and mouse IgG1 constant regions. Anti-B7-H4 light chain variable domains were cloned into expression vectors with mouse IgK constant region. Upon sequence verification, the isolated antibodies were assigned IDs J511-J516 as specified in Table 1 above.

* * *

[0243] The invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

[0244] All references (*e.g.*, publications or patents or patent applications) cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual reference (*e.g.*, publication or patent or patent application) was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[0245] Other embodiments are within the following claims.

WHAT IS CLAIMED:

1. An isolated antibody or antigen-binding fragment thereof that specifically binds to human B7-H4, comprising a heavy chain variable region (VH) complementarity determining region (CDR) 1 comprising the amino acid sequence of SEQ ID NO:22, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:23, a VH CDR3 comprising the amino acid sequence of SEQ ID NO:24, a light chain variable region (VL) CDR1 comprising the amino acid sequence of SEQ ID NO:25, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:26, 27, or 28, and a VL CDR3 sequence comprising the amino acid sequence of SEQ ID NO:29.

2. The antibody or antigen-binding fragment thereof of claim 1, wherein the antibody or antigen-binding fragment thereof comprises a VH comprising the amino acid sequence of SEQ ID NO:6, 7, or 8.

3. The antibody or antigen-binding fragment thereof of claim 1 or claim 2, wherein the antibody or antigen-binding fragment thereof comprises a VL comprising the amino acid sequence of SEQ ID NO:9, 10, or 11.

4. An isolated antibody or antigen-binding fragment thereof that specifically binds to human B7-H4, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:6, 7, or 8.

5. An isolated antibody or antigen-binding fragment thereof that specifically binds to human B7-H4, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO:9, 10, or 11.

6. An isolated antibody or antigen-binding fragment thereof that specifically binds to human B7-H4, comprising a heavy chain variable region and a light chain variable region comprising the amino acid sequences of:

(a) SEQ ID NOs:6 and 9, respectively;
(b) SEQ ID NOs:6 and 10, respectively;
(c) SEQ ID NOs:6 and 11, respectively;
(d) SEQ ID NOs:7 and 9, respectively;
(e) SEQ ID NOs:7 and 10, respectively;
(f) SEQ ID NOs:7 and 11, respectively;
(g) SEQ ID NOs:8 and 9, respectively;
(h) SEQ ID NOs:8 and 10, respectively; or
(i) SEQ ID NOs:8 and 11, respectively.

7. The antibody or antigen-binding fragment thereof of any one of claims 1-6, wherein the antibody or antigen-binding fragment further comprises a heavy chain constant region.

8. The antibody or antigen-binding fragment thereof of claim 7, wherein the heavy chain constant region is a murine IgG_1 or IgG_{2a} heavy chain constant region.

9. The antibody or antigen-binding fragment thereof of any one of claims 1-8, wherein the antibody or antigen-binding fragment further comprises a light chain constant region.

10. The antibody or antigen-binding fragment thereof of claim 9, wherein the light chain constant region is a murine IgGκ light chain constant region.

11. The antibody or antigen-binding fragment thereof of any one of claims 1-6, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:16, 17, 18, 89, 90, or 91.

12. The antibody or antigen-binding fragment thereof of any one of claims 1-6 or 11, wherein the antibody or antigen-binding fragment thereof comprises a light chain comprising the amino acid sequence of SEQ ID NO:19, 30, or 31.

13. The antibody or antigen-binding fragment thereof of any one of claims 1-6, wherein the antibody or antigen-binding fragment comprises a heavy chain and a light chain comprising the amino acid sequences of:

(a) SEQ ID NOs:16 and 19, respectively;
(b) SEQ ID NOs:16 and 30, respectively;
(c) SEQ ID NOs:16 and 31, respectively;
(d) SEQ ID NOs:17 and 19, respectively;
(e) SEQ ID NOs:17 and 30, respectively;
(f) SEQ ID NOs:17 and 31, respectively;
(g) SEQ ID NOs:18 and 19, respectively;
(h) SEQ ID NOs:18 and 30, respectively;
(i) SEQ ID NOs:18 and 31, respectively;
(j) SEQ ID NOs: 89 and 19, respectively;
(k) SEQ ID NOs: 90 and 19, respectively; or
(l) SEQ ID NOs: 91 and 19, respectively.

14. An isolated antibody or antigen-binding fragment thereof that specifically binds to human B7-H4, wherein the antibody or antigen-binding fragment thereof comprises the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 of an antibody selected from the group consisting of J512, J513, J514, J515, J516, J517, J518, J519, J520, J521, and J522.

15. The antibody or antigen-binding fragment thereof of claim 14, wherein the CDRs are the Kabat-defined CDRs, the Chothia-defined CDRs, or the AbM-defined CDRs.

16. An isolated antibody or antigen-binding fragment thereof that specifically binds to the same epitope of human B7-H4 as the antibody or antigen-binding fragment thereof of any one of claims 1-15.

17. An isolated antibody or antigen-binding fragment thereof that competitively inhibits binding of the antibody or antigen-binding fragment thereof of any one of claims 1-15 to human B7-H4.

18. The antibody or antigen-binding fragment thereof of any one of claims 1-17, wherein the antibody or antigen-binding fragment thereof is a murine antibody or antigen-binding fragment thereof.

19. The antibody or antigen binding fragment thereof of any one of claims 1-18, which is a full length antibody.

20. The antibody or antigen binding fragment thereof of any one of claims 1-18, which is an antigen binding fragment.

21. The antigen binding fragment of claim 20, wherein the antigen binding fragment comprises a Fab, Fab', $F(ab')_2$, single chain Fv (scFv), disulfide linked Fv, minibody, $F(ab')_3$, diabody, $(scFv)_2$, or scFv-Fc.

22. The antibody or antigen-binding fragment thereof of any one of claims 1-21, further comprising a detectable label.

23. An isolated polynucleotide comprising a nucleic acid molecule encoding the heavy chain variable region or heavy chain of the antibody or antigen-biding fragment thereof of any one of claims 1-22.

24. The isolated polynucleotide of claim 23, wherein the nucleic acid molecule encodes the VH of SEQ ID NO:6, 7, or 8 or the heavy chain of SEQ ID NO:16, 17, or 18.

25. The isolated polynucleotide of claim 24, wherein the nucleic acid molecule comprises the sequence of SEQ ID NO:12, 13, 14, 93, 94, or 95.

26. An isolated polynucleotide comprising a nucleic acid molecule encoding the light chain variable region or light chain of the antibody or antigen-biding fragment thereof of any one of claims 1-22.

27. The isolated polynucleotide of claim 26, wherein the nucleic acid molecule encodes the VL of SEQ ID NO:9, 10, or 11 or the light chain of SEQ ID NO:19, 30, or 31.

28. The isolated polynucleotide of claim 27, wherein the nucleic acid molecule comprises the sequence of SEQ ID NO:15, 96, or 97.

29. An isolated polynucleotide comprising a nucleic acid molecule encoding the heavy chain variable region or heavy chain of the antibody or antigen-binding fragment thereof of any one of claims 1-22 and the light chain variable region or light chain of the antibody or antigen-binding fragment thereof of any one of claims 1-22.

30. An isolated vector comprising the polynucleotide of any one of claims 23-29.

31. A host cell comprising the polynucleotide of any one of claims 23-29, the vector of claim 30, or a first vector comprising the polynucleotide of any one of claims 23-25 and a second vector comprising the polynucleotide of any one of claims 26-28.

32. The host cell of claim 31, which is a CHO or HEK cell.

33. A method of producing an antibody or antigen-binding fragment thereof that specifically binds to human B7-H4 comprising culturing the host cell of claim 31 or 32 so that the nucleic acid molecule is expressed and the antibody or antigen-biding fragment thereof is produced.

34. An isolated antibody or antigen-binding fragment thereof that specifically binds to human B7-H4 and is encoded by the polynucleotide of any one of claims 23-29.

35. A method for detecting B7-H4 in a sample comprising contacting the sample with the antibody or antigen-binding fragment thereof of any one of claims 1-22 or 34, optionally wherein the method further comprises detecting binding of the antibody or antigen-binding fragment thereof to B7-H4.

36. The method of claim 35, wherein the sample is obtained from a cancer in a subject.

37. The method of claim 36, further comprising administering a therapeutic anti-B7-H4 antibody or antigen-binding fragment thereof to the subject after B7-H4 has been detected.

38. A method of treating a B7-H4 expressing cancer in a subject, the method comprising administering to the subject a therapeutic anti-B7-H4 antibody or antigen-binding fragment thereof, wherein B7-H4 expression was detected in a sample obtained from the subject using the antibody or antigen-binding fragment thereof of any one of claims 1-21 or 33.

39. The method of claim 38, further comprising detecting the B7-H4 in the sample obtained from the cancer.

40. The method of any one of claims 35-39, wherein the detected B7-H4 is cell membrane B7-H4.

41. The method of any one of claims 35-39, wherein the detected B7-H4 is cytoplasmic B7-H4.

42. The method of any one of claims 35-39, wherein B7-H4 is detected in whole cells.

43. The method of any one of claims 35-42, wherein the B7-H4 is detected on circulating tumor cells.

44. The method of any one of claims 35-43, wherein the sample is solid tissue, biopsy, ascites, blood plasma, serum, lymph fluid, or a tumor from a subject.

45. The method of any one of claims 36-44, wherein the cancer is selected from the group consisting of breast cancer, ductal carcinoma, endometrial carcinoma, ovarian cancer,

non-small cell lung cancer, pancreatic cancer, thyroid cancer, kidney cancer and bladder cancer.

46. The method of claim 45, wherein the breast cancer is triple negative breast cancer or wherein the non-small cell lung cancer is squamous cell carcinoma.

47. The method of any one of claims 35-46, wherein the method of detection uses an enzyme linked immunosorbent assay (ELISA), a fluorescence-activated cell sorter (FACS) assay, or immunohistochemistry (IHC).

48. The method of claim 47, wherein the method of detection uses IHC and the concentration of the antibody or antigen-binding fragment thereof of any one of claims 1-21 or 34 is about 1 to about 50 μg/ml.

49. The method of claim 48, wherein the concentration of the antibody or antigen-binding fragment thereof is about 1 to about 20 μg/ml.

50. The method of claim 49, wherein the concentration of the antibody or antigen-binding fragment thereof is about 10 μg/mL.

51. The method of any one of claims 47-50, wherein the method of detection uses IHC and 3,3'-diaminobenzidine (DAB) is used for detection

52. The method of any one of claims 36-51, wherein the subject is human.

53. A kit comprising the antibody or antigen-binding fragment thereof of any one of claims 1-21 or 34 and a) a detection reagent, b) a B7-H4 antigen, c) a therapeutic anti-B7-H4 antibody, or d) a combination of any of (a) through (c).

54. The method or kit of any one of claims 37-53, wherein the therapeutic antibody or antigen-binding fragment comprises the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 of 20502 or 22213.

55. The method or kit of claim 54, wherein the CDRs are the Kabat-defined CDRs, the Chothia-defined CDRs, or the AbM-defined CDR.

56. The method or kit of claim 55, wherein the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 comprise the amino acid sequences of SEQ ID NOs:32-37, respectively or the amino acid sequences of SEQ ID NOs:58-63, respectively.

57. The method or kit of any one of claims 37-56, wherein the therapeutic antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:54 or SEQ ID NO:64.

58. The method or kit of any one of claims 37-56, wherein the therapeutic antibody or antigen-binding fragment comprises a heavy chain variable region and a light chain variable region, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO:55 or SEQ ID NO:65.

59. The method or kit of any one of claims 37-56, wherein the therapeutic antibody or antigen-binding fragment comprises (i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:54 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:55 or (ii) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:64 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:65.

60. The method or kit of any one of claims 37-56, wherein the therapeutic antibody or antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:56 or SEQ ID NO:74.

61. The method or kit of any one of claims 37-56, wherein the therapeutic antibody or antigen-binding fragment thereof comprises a light chain comprising the amino acid sequence of SEQ ID NO:57 or SEQ ID NO:75.

62. The method or kit of any one of claims 37-56, wherein the therapeutic antibody or antigen-binding fragment thereof comprises (i) a heavy chain comprising the amino acid sequence of SEQ ID NO:56 and a light chain comprising the amino acid sequence of SEQ ID NO:57 or (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO:74 and a light chain comprising the amino acid sequence of SEQ ID NO:75.

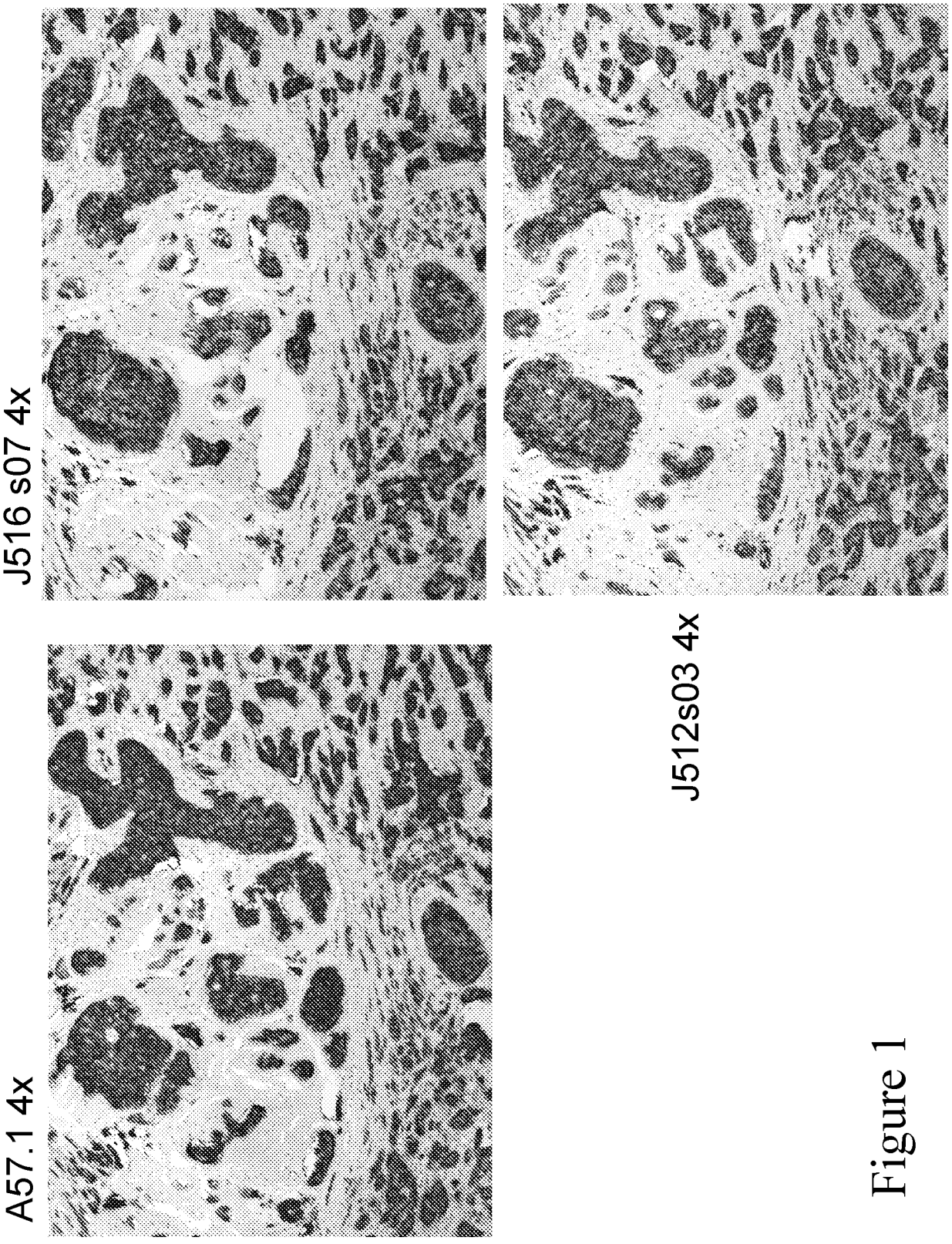


Figure 1

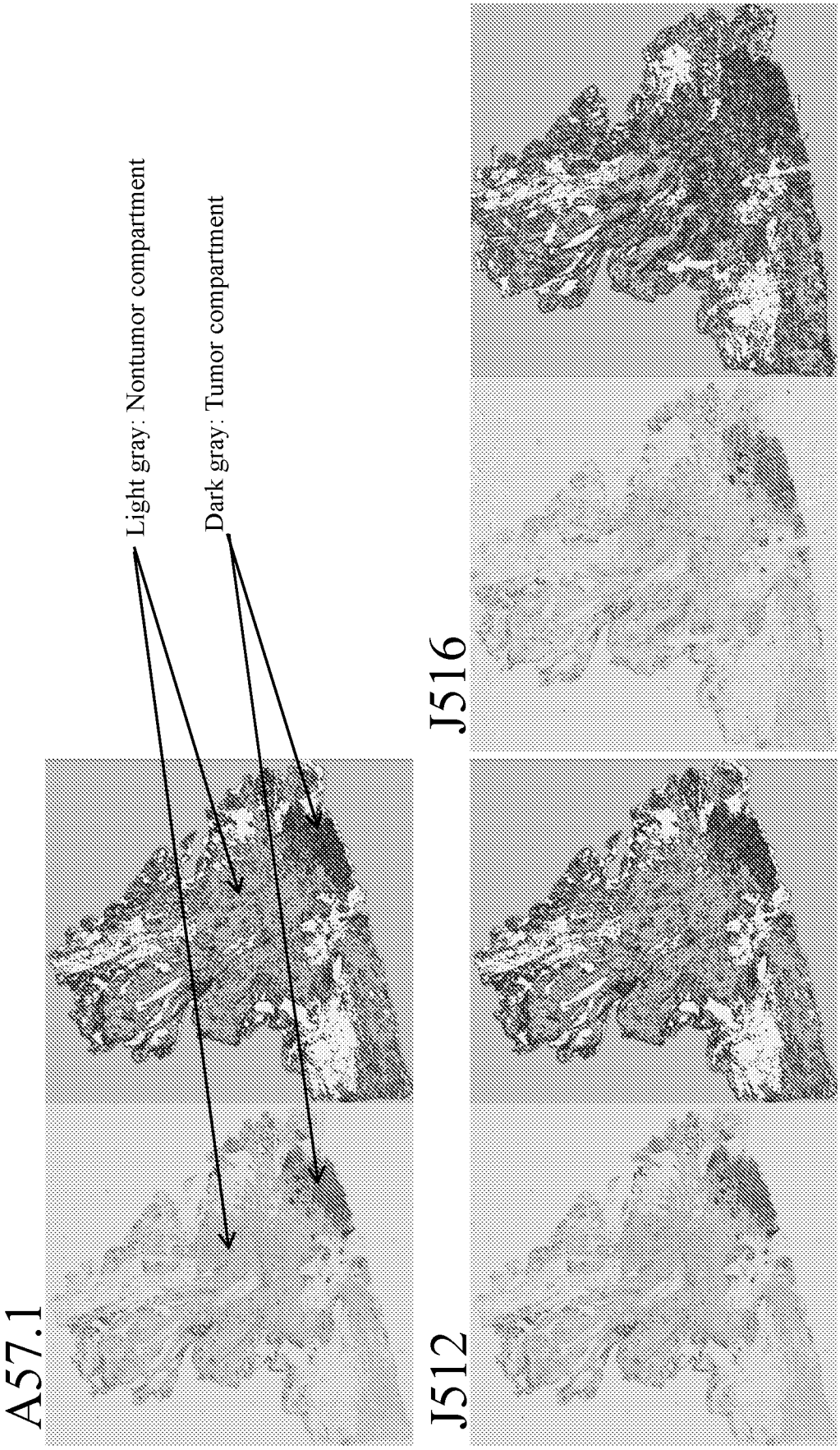

A57.1
J512
J516
Light gray: Nontumor compartment
Dark gray: Tumor compartment

Figure 2

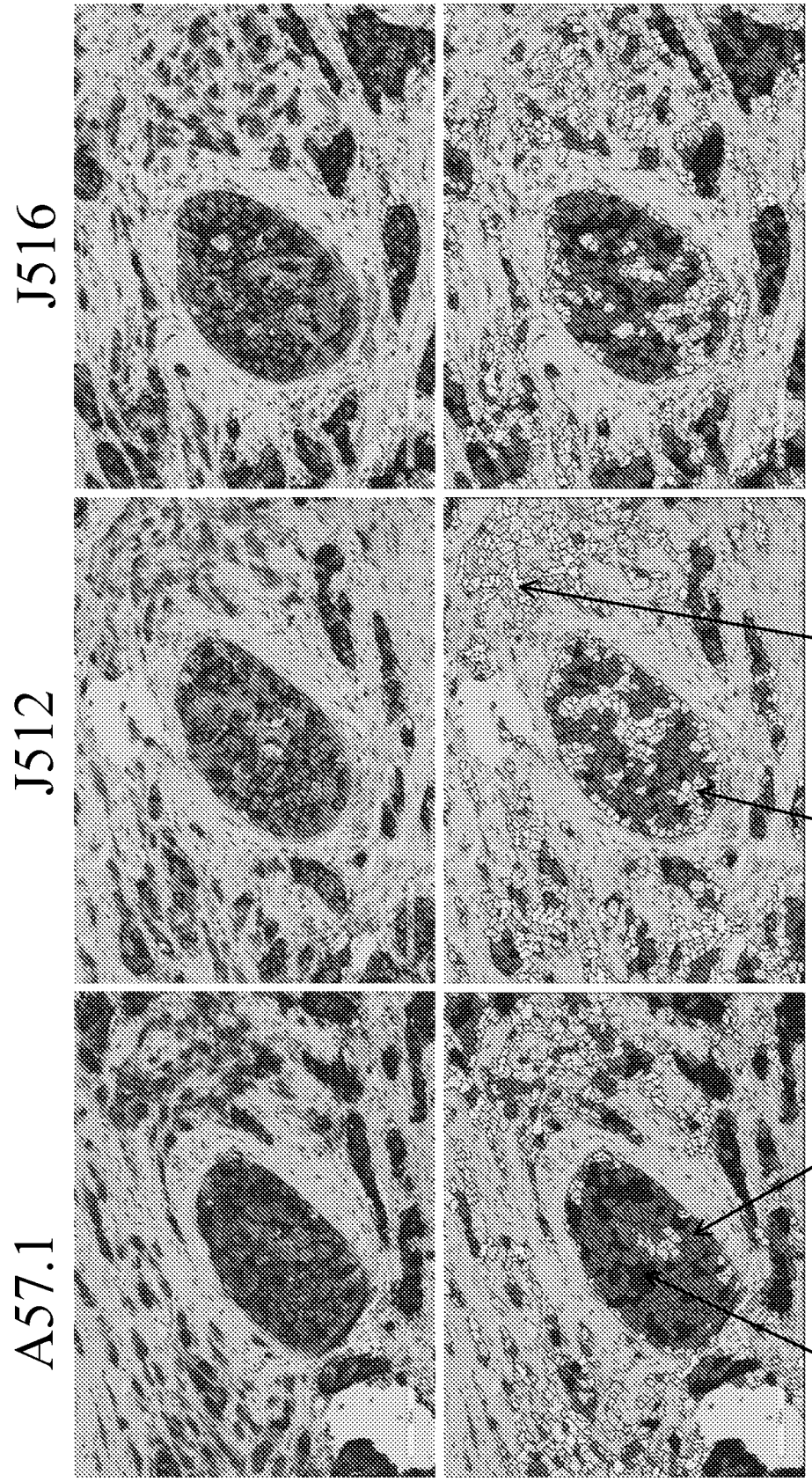
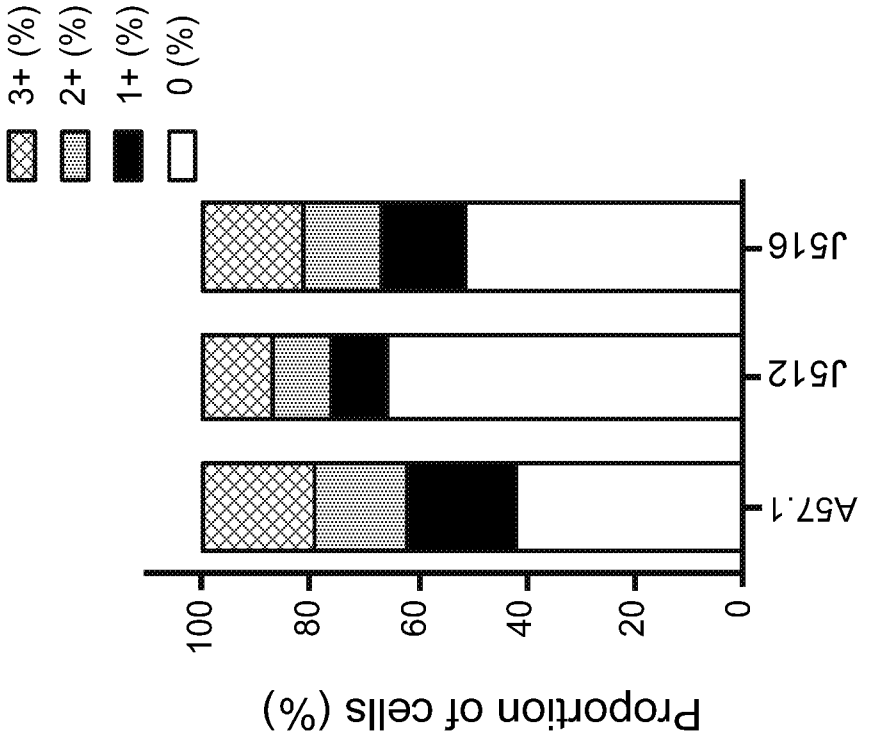
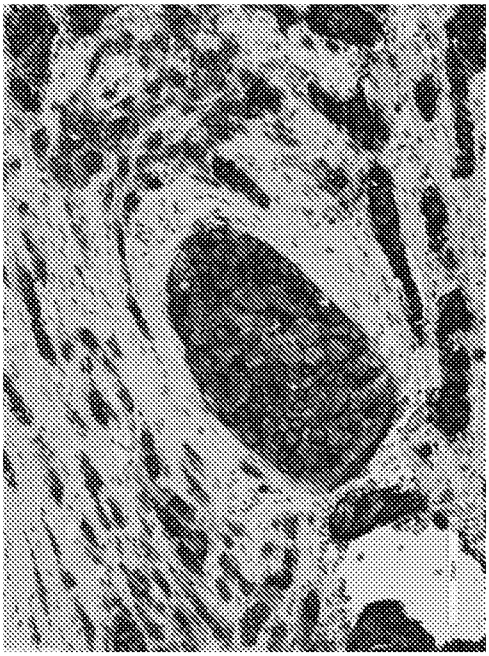


Figure 3



	Total cells	# 0	# 1+	# 2+	# 3+
A57.1	83790	35189	16860	14625	17116
J512	93213	61048	10357	9870	11938
J516	69513	35627	10894	9917	13075

Figure 4



H score (A57.1)=116

Distribution of DAB intensity in B7-H4 positive cells

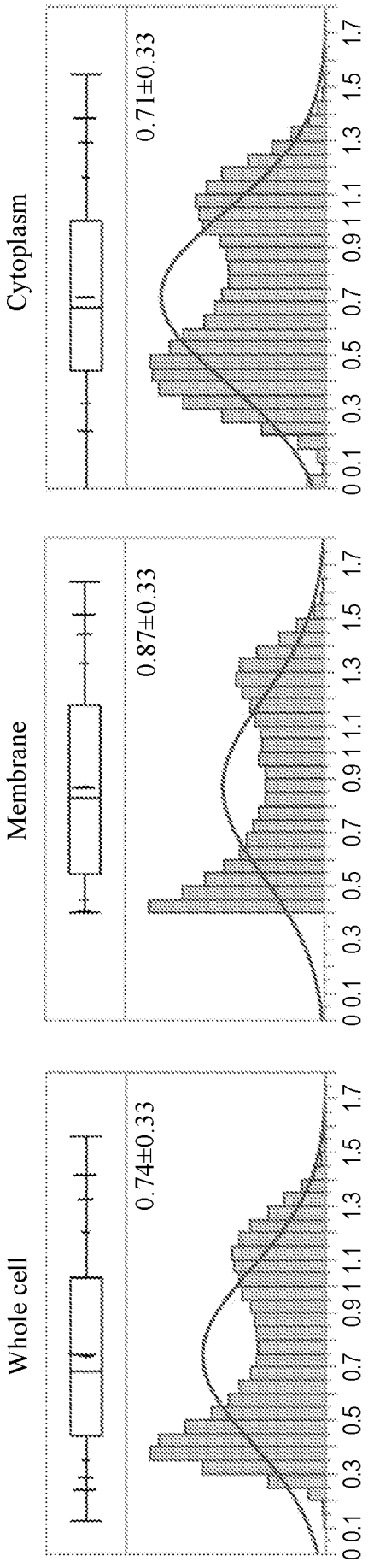
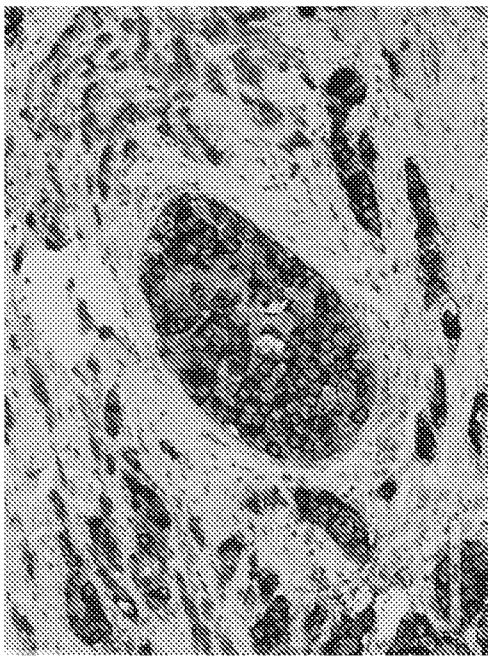


Figure 5A



H score (J512)=71

Distribution of DAB intensity in B7-H4 positive cells

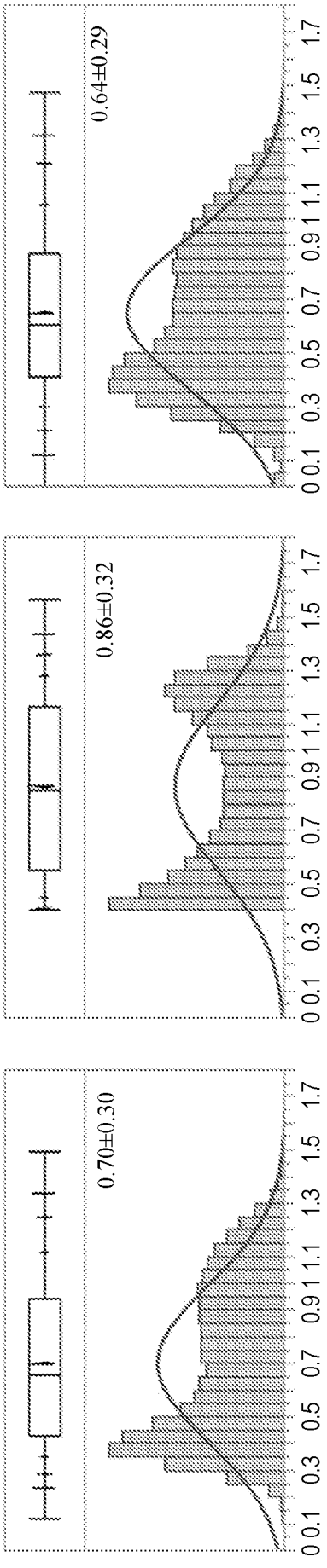
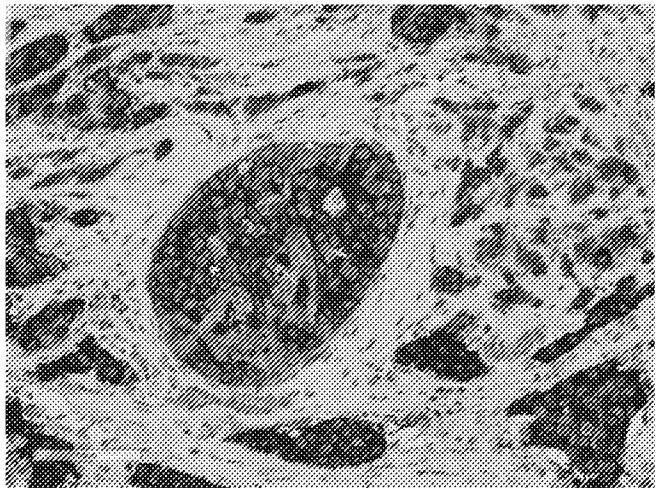


Figure 5B

H score (J516)=101



Distribution of DAB intensity in B7-H4 positive cells

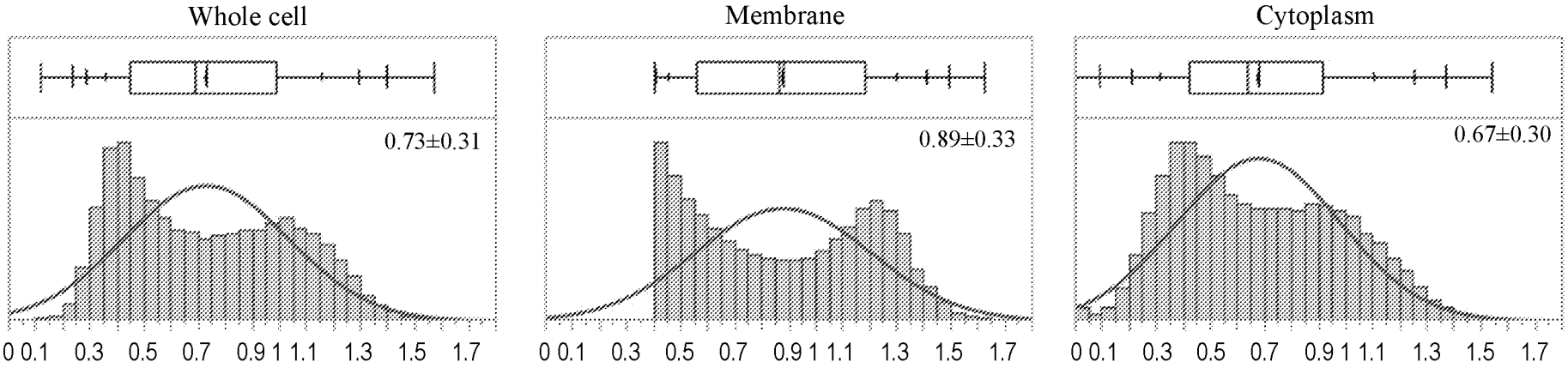


Figure 5C