

ANTI-CD73 ANTIBODIES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

5 [0001] This application claims priority to U.S. Provisional Application No. 62/640,955, filed March 9, 2018; U.S. Provisional Application No. 62/721,044, filed August 22, 2018; and U.S. Provisional Application No. 62/786,598, filed December 31, 2018. Each disclosure is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

10 [0002] This invention relates to monoclonal anti-CD73 antibodies, nucleic acids and expression vectors encoding the antibodies, recombinant cells containing the vectors, and compositions comprising the antibodies. Methods of making the antibodies, and methods of using the antibodies to treat diseases associated with CD73 such as cancer and inflammatory diseases and/or associated complications are also provided.

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REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0003] This application contains a sequence listing, which is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name "689204.10WO Sequence Listing" and a creation date of February 24, 2019 and having a size of 114 kb.

20 The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0004] CD73, also known as ecto-5'-nucleotidase (ecto-5'-NT or EC 3.1.3.5), is a cell surface phosphatase that catalyzes the dephosphorylation of extracellular AMP to produce adenosine. Although it's a glycosphosphatidylinositol (GPI)-anchored protein, CD73 can also be shed to yield a catalytically active form (Arias et al., J Cell Biol. 1997; 136(2):421-31). Physiologically, CD73 is induced by hypoxia to control inflammation at injury sites (Bours et al., Pharmacol Ther 2006; 112:358-404). Pathologically, CD73 is often found

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30 overexpressed on regulatory T cells (Tregs) and tumor cells (Paul et al., Trends Immunol

2012; 33:231-237). Elevated CD73 activity leads to the accumulation of adenosine in the tumor microenvironment (TME).

[0005] Accumulating evidence indicates that CD73 activity in tumor sites is one of the major factors shaping a pro-tumor TME that is critical for tumor growth and survival (Whiteside, Expert Rev. Anticancer Ther. 2017; 17(6):527-35). Extracellular ATP-adenosine homeostasis is determined by the activities of CD39, which converts ATP to AMP, and CD73, which uses AMP to produce adenosine. Through binding to adenosine receptors A2a and A2b, adenosine suppresses both innate and adaptive immunities by regulating many immune cells such as macrophages (Csoka et al., FASEB J 2012; 26:376-386), dendritic cells (Panther et al., Blood 2003; 101:3985-3990), natural killer cells (Hausler et al., Cancer Immunol Immunother 2011; 60:1405-1418), and T effector cells (Hoskin et al., Int J Oncol 2008; 32:527-535). Therefore, it has been hypothesized that immune suppression by adenosine may be alleviated by inhibiting the enzymatic activity of CD73 in the TME. Indeed, *in vivo* animal studies (Jin et al., Cancer Res 2010; 70:2245-2255; Stagg et al., Cancer Res 2011; 71:2890-2900) indicate that inhibiting the enzymatic activity of CD73 suppresses tumor formation and growth, suggesting that CD73 is a promising target for cancer therapy. Monoclonal antibodies that inhibit CD73 enzymatic activity and/or reduce CD73 content on cell surface (i.e., by inducing CD73 internalization) can be efficacious in treating cancer alone as monotherapy or in combination with other immuno-oncology drugs and/or other types of anti-cancer therapies.

BRIEF SUMMARY OF THE INVENTION

[0006] In one general aspect, the invention relates to isolated monoclonal antibodies or antigen-binding fragments thereof that bind CD73.

[0007] Provided are isolated monoclonal antibodies or antigen-binding fragments thereof comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

- (1) SEQ ID NOs:89, 90, 91, 149, 150 and 151, respectively;
- (2) SEQ ID NOs:41, 42, 43, 101, 102 and 103, respectively;
- (3) SEQ ID NOs:44, 45, 46, 104, 105 and 106, respectively;

- (4) SEQ ID NOs:47, 48, 49, 107, 108 and 109, respectively;
- (5) SEQ ID NOs:50, 51, 52, 110, 111 and 112, respectively;
- (6) SEQ ID NOs:53, 54, 55, 113, 114 and 115, respectively;
- (7) SEQ ID NOs:56, 57, 58, 116, 117 and 118, respectively;
- 5 (8) SEQ ID NOs:59, 60, 61, 119, 120 and 121, respectively;
- (9) SEQ ID NOs:62, 63, 64, 122, 123 and 124, respectively;
- (10) SEQ ID NOs:65, 66, 67, 125, 126 and 127, respectively;
- (11) SEQ ID NOs:68, 69, 70, 128, 129 and 130, respectively;
- (12) SEQ ID NOs:71, 72, 73, 131, 132 and 133, respectively;
- 10 (13) SEQ ID NOs:74, 75, 76, 134, 135 and 136, respectively;
- (14) SEQ ID NOs:77, 78, 79, 137, 138 and 139, respectively;
- (15) SEQ ID NOs:80, 81, 82, 140, 141 and 142, respectively;
- (16) SEQ ID NOs:83, 84, 85, 143, 144 and 145, respectively;
- (17) SEQ ID NOs:86, 87, 88, 146, 147 and 148, respectively;
- 15 (18) SEQ ID NOs:92, 93, 94, 152, 153 and 154, respectively;
- (19) SEQ ID NOs:95, 96, 97, 155, 156 and 157, respectively; or
- (20) SEQ ID NOs:98, 99, 100, 158, 159 and 160, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds to CD73, preferably human CD73.

- 20 **[0008]** Provided are isolated monoclonal antibodies or antigen-binding fragments thereof comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

- (1) SEQ ID NOs:209, 210, 211, 269, 270 and 271, respectively;
- 25 (2) SEQ ID NOs:161, 162, 163, 221, 222 and 223, respectively;
- (3) SEQ ID NOs:164, 165, 166, 224, 225 and 226, respectively;
- (4) SEQ ID NOs:167, 168, 169, 227, 228 and 229, respectively;
- (5) SEQ ID NOs:170, 171, 172, 230, 231 and 232, respectively;
- (6) SEQ ID NOs:173, 174, 175, 233, 234 and 235, respectively;
- 30 (7) SEQ ID NOs:176, 177, 178, 236, 237 and 238, respectively;
- (8) SEQ ID NOs:179, 180, 181, 239, 240 and 241, respectively;

- (9) SEQ ID NOs:182, 183, 184, 242, 243 and 244, respectively;
 (10) SEQ ID NOs:185, 186, 187, 245, 246 and 247, respectively;
 (11) SEQ ID NOs:188, 189, 190, 248, 249 and 250, respectively;
 (12) SEQ ID NOs:191, 192, 193, 251, 252 and 253, respectively;
 5 (13) SEQ ID NOs:194, 195, 196, 254, 255 and 256, respectively;
 (14) SEQ ID NOs:197, 198, 199, 257, 258 and 259, respectively;
 (15) SEQ ID NOs:200, 201, 202, 260, 261 and 262, respectively;
 (16) SEQ ID NOs:203, 204, 205, 263, 264 and 265, respectively;
 (17) SEQ ID NOs:206, 207, 208, 266, 267 and 268, respectively;
 10 (18) SEQ ID NOs:212, 213, 214, 272, 273 and 274, respectively;
 (19) SEQ ID NOs:215, 216, 217, 275, 276 and 277, respectively; or
 (20) SEQ ID NOs:218, 219, 220, 278, 279 and 280, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds to CD73, preferably human CD73.

- 15 **[0009]** In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:33, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 35, 37 or 39, or a light chain variable region having a polypeptide sequence at least 95%, at least 96%, at least
 20 97%, at least 98%, or at least 99% identical to SEQ ID NO:34, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 36, 38 or 40.

[0010] In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment thereof comprises:

- (a) a heavy chain variable region having the polypeptide sequence of SEQ ID
 25 NO:33, and a light chain variable region having the polypeptide sequence of SEQ ID NO:34;
 (b) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;

- (c) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;
- 5 (d) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;
- (e) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;
- 10 (f) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;
- (g) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;
- 15 (h) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;
- (i) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;
- 20 (j) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18;
- 25 (k) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20;
- (l) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21, and a light chain variable region having the polypeptide sequence of SEQ ID NO:22;
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- (m) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23, and a light chain variable region having the polypeptide sequence of SEQ ID NO:24;
- 5 (n) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:25, and a light chain variable region having the polypeptide sequence of SEQ ID NO:26;
- (o) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:27, and a light chain variable region having the polypeptide sequence of SEQ ID NO:28;
- 10 (p) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:29, and a light chain variable region having the polypeptide sequence of SEQ ID NO:30;
- (q) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:31, and a light chain variable region having the polypeptide sequence of SEQ ID NO:32;
- 15 (r) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:35, and a light chain variable region having the polypeptide sequence of SEQ ID NO:36;
- (s) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:37, and a light chain variable region having the polypeptide sequence of SEQ ID NO:38; or
- 20 (t) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:39, and a light chain variable region having the polypeptide sequence of SEQ ID NO:40.
- 25 **[0011]** In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment thereof inhibits the enzyme activity of soluble and/or cell-surface CD73.
- [0012]** In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment thereof prevents the dimerization of CD73.
- [0013]** In certain embodiments, the isolated monoclonal antibody or antigen-binding
- 30 fragment thereof induces the internalization of CD73.

[0014] In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment thereof is chimeric.

[0015] In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment thereof is human or humanized.

5 **[0016]** In certain embodiments, the humanized monoclonal antibody or antigen-binding fragment thereof comprises:

- (1) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:286, and a light chain variable region having the polypeptide sequence of SEQ ID NO:293;
- 10 (2) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:282, and a light chain variable region having the polypeptide sequence of SEQ ID NO:290;
- (3) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:282, and a light chain variable region having the polypeptide sequence of
- 15 SEQ ID NO:291;
- (4) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:282, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;
- (5) a heavy chain variable region having the polypeptide sequence of SEQ ID
- 20 NO:283, and a light chain variable region having the polypeptide sequence of SEQ ID NO:290;
- (6) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:283, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;
- 25 (7) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:283, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;
- (8) a heavy chain variable region having the polypeptide sequence of SEQ ID
- 30 NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:290;

- (9) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;
- 5 (10) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;
- (11) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:285, and a light chain variable region having the polypeptide sequence of SEQ ID NO:290;
- 10 (12) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:285, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;
- (13) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:285, and a light chain variable region having the polypeptide sequence of
- 15 SEQ ID NO:292;
- (14) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:283, and a light chain variable region having the polypeptide sequence of SEQ ID NO:293;
- (15) a heavy chain variable region having the polypeptide sequence of SEQ ID
- 20 NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:293;
- (16) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:285, and a light chain variable region having the polypeptide sequence of SEQ ID NO:293;
- 25 (17) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:286, and a light chain variable region having the polypeptide sequence of SEQ ID NO:290;
- (18) a heavy chain variable region having the polypeptide sequence of SEQ ID
- 30 NO:286, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;

- (19) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:286, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;
- 5 (20) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:287, and a light chain variable region having the polypeptide sequence of SEQ ID NO:294;
- (21) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:288, and a light chain variable region having the polypeptide sequence of SEQ ID NO:294;
- 10 (22) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:289, and a light chain variable region having the polypeptide sequence of SEQ ID NO:294;
- (23) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:284, and a light chain variable region having the polypeptide sequence of
- 15 SEQ ID NO:299;
- (24) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:295, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299;
- (25) a heavy chain variable region having the polypeptide sequence of SEQ ID
- 20 NO:296, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299;
- (26) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:297, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299; or
- 25 (27) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:298, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299.

[0017] In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment thereof is capable of activating T cells.

- 30 **[0018]** Also provided are isolated nucleic acids encoding the monoclonal antibodies or antigen-binding fragments thereof of the invention disclosed herein.

[0019] Also provided are vectors comprising the isolated nucleic acids encoding a monoclonal antibody or antigen-binding fragment thereof of the invention.

[0020] Also provided are host cells comprising the vectors comprising the isolated nucleic acids encoding a monoclonal antibody or antigen-binding fragment thereof of the invention.

[0021] In certain embodiments, provided is a pharmaceutical composition comprising the isolated monoclonal antibody or antigen-binding fragment thereof of the invention and a pharmaceutically acceptable carrier.

[0022] Also provided are methods of inhibiting the nucleotidase activity of CD73 in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the invention.

[0023] Also provided are methods of preventing the dimerization of CD73 in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the invention.

[0024] Also provided are methods of inducing the internalization of CD73 in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the invention.

[0025] Also provided are methods of treating cancer in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the invention.

The cancer can be any liquid or solid cancer, for example, it can be selected from, but not limited to, a lung cancer, a gastric cancer, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

[0026] Also provided are methods of producing the monoclonal antibody or antigen-binding fragment thereof of the invention, comprising culturing a cell comprising a nucleic acid encoding the monoclonal antibody or antigen-binding fragment thereof

under conditions to produce the monoclonal antibody or antigen-binding fragment thereof, and recovering the antibody or antigen-binding fragment thereof from the cell or culture.

[0027] Also provided are methods of producing a pharmaceutical composition comprising the monoclonal antibody or antigen-binding fragment thereof of the invention, comprising combining the monoclonal antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

[0028] Also provided are methods of determining a level of CD73 in a subject. The methods comprise (a) obtaining a sample from the subject; (b) contacting the sample with an antibody or antigen-binding fragment thereof of the invention; and (c) determining the level of CD73 in the subject. In certain embodiments, the sample is a tissue sample. The tissue sample can, for example, be a cancer tissue sample. In certain embodiments, the sample is a blood sample.

[0029] Also provided are methods of determining the ecto-5'-nucleotidase activity of CD73 in a subject, wherein the enzyme activity can be fully inhibited by the monoclonal antibody or antigen-binding fragment thereof of the invention. The methods comprise (a) obtaining a sample from the subject; (b) contacting the sample with an antibody or antigen-binding fragment thereof of the invention; and (c) determining the ecto-5'-nucleotidase activity of CD73 in the subject. In certain embodiments, the sample is a tissue sample. The tissue sample can, for example, be a cancer tissue sample. In certain embodiments, the sample is a blood sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] The foregoing summary, as well as the following detailed description of preferred embodiments of the present application, will be better understood when read in conjunction with the appended drawings. It should be understood, however, that the application is not limited to the precise embodiments shown in the drawings.

[0031] FIGs. 1A and 1B show the inhibition of the nucleotidase activity of human CD73 immobilized on a plate by chimeric anti-CD73 mAbs. No Antibody, enzyme reaction with no antibody added; AMPCP (adenosine 5' (α , β -methylene) diphosphate) was used as a control for inhibition of enzyme activity.

[0032] FIGs. 2A-2E show the binding of chimeric anti-CD73 mAbs to human CD73 in an ELISA assay.

[0033] FIG. 3 shows the inhibition of the nucleotidase activity of soluble human CD73 by chimeric mAbs 60A9A/IgG4 and 39G8A/IgG4. No mAb, enzyme reaction with no antibody added; No CD73, negative control reaction with neither antibody nor CD73 added.

[0034] FIGs. 4A-4D show the binding to human CD73 and inhibition of nucleotidase activity of human CD73 by the humanized mAb 60A9-H5L4/IgG4. FIG. 4A shows the binding of humanized mAb 60A9-H5L4/IgG4 to human CD73 in an ELISA assay;

chimeric mAb 60A9A/IgG4 was used as control. FIG. 4B shows the inhibition of the nucleotidase activity of soluble human CD73 by humanized mAb 60A9-H5L4/IgG4. No mAb, enzyme reaction with no mAb added; No CD73, enzyme reaction with no CD73 added. FIG. 4C shows the inhibition of the nucleotidase activity of human CD73

expressed on the surface of A375 cells by humanized mAb 60A9-H5L4/IgG4; chimeric

mAb 60A9A/IgG4 was used as control. No mAb, enzyme reaction with no mAb added; No AMP, enzyme reaction with no AMP added; No Cells, enzyme reaction with no cells added. FIG. 4D shows the inhibition of the nucleotidase activity of human CD73

expressed on the surface of A375 cells by humanized mAbs 60A9-H3L5/IgG4, 60A9-H5L5/IgG4, 60A9-H6L5/IgG4, 60A9-H7L5/IgG4, 60A9-H8L5/IgG4 and 60A9-

H9L5/IgG4; chimeric mAb 60A9A/IgG4 was used as control. No mAb, enzyme reaction with no mAb added; No AMP, enzyme reaction with no AMP added; No cells, enzyme reaction with no cells added.

[0035] FIG. 5 shows the inhibition of the nucleotidase activity of CD73 in patient serum samples by humanized anti-CD73 mAb 60A9-H5L4/IgG4. No mAb, enzyme reaction with no antibody added; Assay background, enzyme reaction with 100 μ M AMP and 100 μ M ATP added; cps, counts/second.

[0036] FIG. 6 shows the activation of T cell proliferation by humanized anti-CD73 mAb 60A9-H5L4/IgG4.

DETAILED DESCRIPTION OF THE INVENTION

[0037] Various publications, articles and patents are cited or described in the background and throughout the specification; each of these references is herein incorporated by reference in its entirety. Discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is for the purpose of providing context for the invention. Such discussion is not an admission that any or all of these matters form part of the prior art with respect to any inventions disclosed or claimed.

[0038] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention pertains. Otherwise, certain terms used herein have the meanings as set forth in the specification.

[0039] It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise.

[0040] Unless otherwise stated, any numerical values, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term “about.” Thus, a numerical value typically includes $\pm 10\%$ of the recited value. For example, a concentration of 1 mg/mL includes 0.9 mg/mL to 1.1 mg/mL. Likewise, a concentration range of 1% to 10% (w/v) includes 0.9% (w/v) to 11% (w/v). As used herein, the use of a numerical range expressly includes all possible subranges, all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly indicates otherwise.

[0041] Unless otherwise indicated, the term “at least” preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the invention.

[0042] As used herein, the terms “comprises,” “comprising,” “includes,” “including,” “has,” “having,” “contains” or “containing,” or any other variation thereof, will be understood to imply the inclusion of a stated integer or group of integers but not the

exclusion of any other integer or group of integers and are intended to be non-exclusive or open-ended. For example, a composition, a mixture, a process, a method, an article, or an apparatus that comprises a list of elements is not necessarily limited to only those elements but can include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the contrary, “or” refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

10 **[0043]** As used herein, the conjunctive term “and/or” between multiple recited elements is understood as encompassing both individual and combined options. For instance, where two elements are conjoined by “and/or,” a first option refers to the applicability of the first element without the second. A second option refers to the applicability of the second element without the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and therefore satisfy the requirement of the term “and/or” as used herein. Concurrent applicability of more than one of the options is also understood to fall within the meaning, and therefore satisfy the requirement of the term “and/or.”

20 **[0044]** As used herein, the term “consists of,” or variations such as “consist of” or “consisting of,” as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, but that no additional integer or group of integers can be added to the specified method, structure, or composition.

25 **[0045]** As used herein, the term “consists essentially of,” or variations such as “consist essentially of” or “consisting essentially of,” as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, and the optional inclusion of any recited integer or group of integers that do not materially change the basic or novel properties of the specified method, structure or composition. See M.P.E.P. § 2111.03.

30 **[0046]** As used herein, “subject” means any animal, preferably a mammal, most preferably a human. The term “mammal” as used herein, encompasses any mammal.

Examples of mammals include, but are not limited to, cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, monkeys, humans, etc., more preferably a human.

[0047] The words “right,” “left,” “lower,” and “upper” designate directions in the drawings to which reference is made.

5 **[0048]** It should also be understood that the terms “about,” “approximately,” “generally,” “substantially,” and like terms, used herein when referring to a dimension or characteristic of a component of the preferred invention, indicate that the described dimension/characteristic is not a strict boundary or parameter and does not exclude minor variations therefrom that are functionally the same or similar, as would be understood by
10 one having ordinary skill in the art. At a minimum, such references that include a numerical parameter would include variations that, using mathematical and industrial principles accepted in the art (e.g., rounding, measurement or other systematic errors, manufacturing tolerances, etc.), would not vary the least significant digit.

[0049] The terms “identical” or percent “identity,” in the context of two or more
15 nucleic acids or polypeptide sequences (e.g., anti-CD73 antibodies and polynucleotides that encode them, CD73 polypeptides and CD73 polynucleotides that encode them), refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence
20 comparison algorithms or by visual inspection.

[0050] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The
25 sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0051] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the
30 homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat’l. Acad. Sci. USA

85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (see generally, Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, a joint
5 venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1995 Supplement) (Ausubel)).

[0052] Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1990) J. Mol. Biol. 215: 403-410 and Altschul et al. (1997)
10 Nucleic Acids Res. 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a
15 database sequence. T is referred to as the neighborhood word score threshold (Altschul et al, supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased.

[0053] Cumulative scores are calculated using, for nucleotide sequences, the
20 parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the
25 accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as
30 defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)).

[0054] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides
5 an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

10 [0055] A further indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative
15 substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions.

[0056] As used herein, the terms "inhibit," "inhibiting," and "inhibition," mean to decrease an activity, response, condition, disease or other biological parameter. This can include, but is not limited to, complete ablation of the activity, response, condition, or
20 disease. This may also include, for example, a 10% reduction in the activity, response, condition, or disease as compared to the native or control level. Thus, the reduction can be a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between, as compared to native or control levels. By way of a non-limiting example, an antibody of the invention can inhibit the nucleotidase activity of a CD73 protein. The activity of the
25 CD73 protein can be reduced or ablated relative to the native CD73 protein activity. By way of another non-limiting example, an antibody of the invention can inhibit or prevent the dimerization of a CD73 protein in a subject. The dimerization of CD73 can be reduced or ablated relative to the native CD73 dimerization.

[0057] **Antibodies**

30 [0058] The invention generally relates to isolated anti-CD73 antibodies, nucleic acids and expression vectors encoding the antibodies, recombinant cells containing the vectors,

and compositions comprising the antibodies. The invention also generally relates to methods of making the antibodies and methods of using the antibodies to treat diseases, including cancer. The antibodies of the invention possess one or more desirable functional properties, including but not limited to, high-affinity binding to CD73, high specificity to CD73, the ability to inhibit the nucleotidase activity of CD73, the ability to prevent the dimerization of CD73, the ability to induce the internalization of CD73 into cells, which results in a decrease in the cell surface content of CD73, and the ability to inhibit tumor growth in subjects in need thereof and in animal models when administered alone or in combination with other anti-cancer therapies.

10 **[0059]** In a general aspect, the invention relates to isolated monoclonal antibodies or antigen-binding fragments thereof that specifically bind CD73.

[0060] As used herein, the term “antibody” is used in a broad sense and includes immunoglobulin or antibody molecules including human, humanized, composite and chimeric antibodies and antibody fragments that are monoclonal or polyclonal. In general, antibodies are proteins or peptide chains that exhibit binding specificity to a specific antigen. Antibody structures are well known. Immunoglobulins can be assigned to five major classes (i.e., IgA, IgD, IgE, IgG and IgM), depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4. Accordingly, the antibodies of the invention can be of any of the five major classes or corresponding sub-classes. Preferably, the antibodies of the invention are IgG1, IgG2, IgG3 or IgG4. Antibody light chains of vertebrate species can be assigned to one of two clearly distinct types, namely kappa and lambda, based on the amino acid sequences of their constant domains. Accordingly, the antibodies of the invention can contain a kappa or lambda light chain constant domain.

25 According to particular embodiments, the antibodies of the invention include heavy and/or light chain constant regions from rat or human antibodies. In addition to the heavy and light constant domains, antibodies contain an antigen-binding region that is made up of a light chain variable region and a heavy chain variable region, each of which contains three domains (i.e., complementarity determining regions 1-3; CDR1, CDR2, and CDR3).

30 The light chain variable region domains are alternatively referred to as LCDR1, LCDR2,

and LCDR3, and the heavy chain variable region domains are alternatively referred to as HCDR1, HCDR2, and HCDR3.

[0061] As used herein, the term an “isolated antibody” refers to an antibody which is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds to CD73 is substantially free of antibodies that do not bind to CD73). In addition, an isolated antibody is substantially free of other cellular material and/or chemicals.

[0062] As used herein, the term “monoclonal antibody” refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. The monoclonal antibodies of the invention can be made by the hybridoma method, phage display technology, single lymphocyte gene cloning technology, or by recombinant DNA methods. For example, the monoclonal antibodies can be produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, such as a transgenic mouse or rat, having a genome comprising a human heavy chain transgene and a light chain transgene.

[0063] As used herein, the term “antigen-binding fragment” refers to an antibody fragment such as, for example, a diabody, a Fab, a Fab', a F(ab')₂, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)₂, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), a single domain antibody (sdab) an scFv dimer (bivalent diabody), a multispecific antibody formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a bivalent domain antibody, or any other antibody fragment that binds to an antigen but does not comprise a complete antibody structure. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody or a parent antibody fragment binds. According to particular embodiments, the antigen-binding fragment comprises a light chain variable region, a light chain constant region, and an Fd segment of the heavy chain. According to other particular embodiments, the antigen-binding fragment comprises Fab and F(ab').

[0064] As used herein, the term “single-chain antibody” refers to a conventional single-chain antibody in the field, which comprises a heavy chain variable region and a light

chain variable region connected by a short peptide of about 15 to about 20 amino acids. As used herein, the term “single domain antibody” refers to a conventional single domain antibody in the field, which comprises a heavy chain variable region and a heavy chain constant region or which comprises only a heavy chain variable region.

5 **[0065]** As used herein, the term “human antibody” refers to an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human made using any technique known in the art. This definition of a human antibody includes intact or full-length antibodies, fragments thereof, and/or antibodies comprising at least one human heavy and/or light chain polypeptide.

10 **[0066]** As used herein, the term “humanized antibody” refers to a non-human antibody that is modified to increase the sequence homology to that of a human antibody, such that the antigen-binding properties of the antibody are retained, but its antigenicity in the human body is reduced.

15 **[0067]** As used herein, the term “chimeric antibody” refers to an antibody wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. The variable region of both the light and heavy chains often corresponds to the variable region of an antibody derived from one species of mammal (e.g., mouse, rat, rabbit, etc.) having the desired specificity, affinity, and capability, while the constant regions correspond to the sequences of an antibody derived from another species of mammal (e.g., human) to avoid eliciting an immune response in that species.

20 **[0068]** As used herein, the term “multispecific antibody” refers to an antibody that comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In an embodiment, the first and second epitopes are on the same antigen, e.g., the same protein (or subunit of a multimeric protein). In an embodiment, the first and second epitopes overlap or substantially overlap. In an embodiment, the first and second epitopes do not overlap or do not substantially overlap. In an embodiment, the first and second epitopes are on different antigens, e.g., different proteins (or different subunits of a multimeric protein). In an embodiment, a
25 multispecific antibody comprises a third, fourth, or fifth immunoglobulin variable domain.

In an embodiment, a multispecific antibody is a bispecific antibody molecule, a trispecific antibody molecule, or a tetraspecific antibody molecule.

[0069] As used herein, the term “bispecific antibody” refers to a multispecific antibody that binds no more than two epitopes or two antigens. A bispecific antibody is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In an embodiment, the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment, the first and second epitopes overlap or substantially overlap. In an embodiment, the first and second epitopes are on different antigens, *e.g.*, different proteins (or different subunits of a multimeric protein). In an embodiment, a bispecific antibody comprises a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a first epitope and a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a second epitope. In an embodiment, a bispecific antibody comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment, a bispecific antibody comprises a scFv, or fragment thereof, having binding specificity for a first epitope, and a scFv, or fragment thereof, having binding specificity for a second epitope. In an embodiment, the first epitope is located on CD73 and the second epitope is located on PD-1, PD-L1, TIM-3, LAG-3, CD47, CD3, apelin, claudin18.2, DLL3, folate receptor alpha (FR α), TIP-1, CTLA-4, EGFR, HER-2, CD19, CD20, CD33 and/or other tumor associated immune suppressors or surface antigens.

[0070] As used herein, the term “CD73” refers to the ecto-5'-nucleotidase (ecto-5'-NT or EC 3.1.3.5), a cell surface phosphatase that catalyzes the dephosphorylation of extracellular AMP to produce adenosine. Physiologically, CD73 is induced by hypoxia to control inflammation at injury sites (Bours et al., *Pharmacol Ther* 2006; 112:358-404). Pathologically, CD73 is often found overexpressed on regulatory T cells (Tregs) and tumor cells (Paul et al., *Trends Immunol* 2012; 33:231-237). The elevated CD73 activity leads to the accumulation of adenosine in the tumor microenvironment (TME). Through

binding to adenosine receptors A2a and A2b, adenosine suppresses both innate and adaptive immunities by regulating many immune cells such as macrophages (Csoka et al., FASEB J 2012; 26:376-386), dendritic cells (Panther et al., Blood 2003; 101:3985-3990), natural killer cells (Hausler et al., Cancer Immunol Immunother 2011; 60:1405-1418),
5 and T effector cells (Hoskin et al., Int J Oncol 2008; 32:527-535). Therefore, it has been postulated that immune suppression by adenosine may be alleviated by inhibiting the activity of CD73 in the TME. Indeed, *in vivo* animal studies (Jin et al., Cancer Res 2010; 70:2245-2255; Stagg et al., Cancer Res 2011; 71:2890-2900) indicate that inhibiting the activity of CD73 inhibits tumor formation and growth, suggesting that CD73 is a
10 promising target for cancer therapy. The term "human CD73" refers to a CD73 originated from a human. An exemplary amino acid sequence of a human CD73 is represented in GenBank Accession No. P21589.1 (SEQ ID NO:281).

[0071] As used herein, an antibody that "specifically binds to CD73" refers to an antibody that binds to a CD73, preferably a human CD73, with a KD of 1×10^{-7} M or less,
15 preferably 1×10^{-8} M or less, more preferably 5×10^{-9} M or less, 1×10^{-9} M or less, 5×10^{-10} M or less, or 1×10^{-10} M or less. The term "KD" refers to the dissociation constant, which is obtained from the ratio of Kd to Ka (i.e., Kd/Ka) and is expressed as a molar concentration (M). KD values for antibodies can be determined using methods in the art in view of the present disclosure. For example, the KD of an antibody can be determined
20 by using surface plasmon resonance, such as by using a biosensor system, e.g., a Biacore® system, or by using bio-layer interferometry technology, such as an Octet RED96 system.

[0072] The smaller the value of the KD of an antibody, the higher affinity that the antibody binds to a target antigen.

25 **[0073]** According to a particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, a HCDR3, a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3, having the polypeptide sequences of:

- 30 (1) SEQ ID NOs:89, 90, 91, 149, 150 and 151, respectively;
(2) SEQ ID NOs:41, 42, 43, 101, 102 and 103, respectively;

- (3) SEQ ID NOs:44, 45, 46, 104, 105 and 106, respectively;
- (4) SEQ ID NOs:47, 48, 49, 107, 108 and 109, respectively;
- (5) SEQ ID NOs:50, 51, 52, 110, 111 and 112, respectively;
- (6) SEQ ID NOs:53, 54, 55, 113, 114 and 115, respectively;
- 5 (7) SEQ ID NOs:56, 57, 58, 116, 117 and 118, respectively;
- (8) SEQ ID NOs:59, 60, 61, 119, 120 and 121, respectively;
- (9) SEQ ID NOs:62, 63, 64, 122, 123 and 124, respectively;
- (10) SEQ ID NOs:65, 66, 67, 125, 126 and 127, respectively;
- (11) SEQ ID NOs:68, 69, 70, 128, 129 and 130, respectively;
- 10 (12) SEQ ID NOs:71, 72, 73, 131, 132 and 133, respectively;
- (13) SEQ ID NOs:74, 75, 76, 134, 135 and 136, respectively;
- (14) SEQ ID NOs:77, 78, 79, 137, 138 and 139, respectively;
- (15) SEQ ID NOs:80, 81, 82, 140, 141 and 142, respectively;
- (16) SEQ ID NOs:83, 84, 85, 143, 144 and 145, respectively;
- 15 (17) SEQ ID NOs:86, 87, 88, 146, 147 and 148, respectively;
- (18) SEQ ID NOs:92, 93, 94, 152, 153 and 154, respectively;
- (19) SEQ ID NOs:95, 96, 97, 155, 156 and 157, respectively; or
- (20) SEQ ID NOs:98, 99, 100, 158, 159 and 160, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds to CD73,
 20 preferably human CD73.

[0074] According to a particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, a HCDR3, a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3, having the polypeptide
 25 sequences of:

- (1) SEQ ID NOs:209, 210, 211, 269, 270 and 271, respectively;
- (2) SEQ ID NOs:161, 162, 163, 221, 222 and 223, respectively;
- (3) SEQ ID NOs:164, 165, 166, 224, 225 and 226, respectively;
- (4) SEQ ID NOs:167, 168, 169, 227, 228 and 229, respectively;
- 30 (5) SEQ ID NOs:170, 171, 172, 230, 231 and 232, respectively;
- (6) SEQ ID NOs:173, 174, 175, 233, 234 and 235, respectively;

- (7) SEQ ID NOs:176, 177, 178, 236, 237 and 238, respectively;
(8) SEQ ID NOs:179, 180, 181, 239, 240 and 241, respectively;
(9) SEQ ID NOs:182, 183, 184, 242, 243 and 244, respectively;
(10)SEQ ID NOs:185, 186, 187, 245, 246 and 247, respectively;
5 (11)SEQ ID NOs:188, 189, 190, 248, 249 and 250, respectively;
(12)SEQ ID NOs:191, 192, 193, 251, 252 and 253, respectively;
(13)SEQ ID NOs:194, 195, 196, 254, 255 and 256, respectively;
(14)SEQ ID NOs:197, 198, 199, 257, 258 and 259, respectively;
(15)SEQ ID NOs:200, 201, 202, 260, 261 and 262, respectively;
10 (16)SEQ ID NOs:203, 204, 205, 263, 264 and 265, respectively;
(17)SEQ ID NOs:206, 207, 208, 266, 267 and 268, respectively;
(18)SEQ ID NOs:212, 213, 214, 272, 273 and 274, respectively;
(19)SEQ ID NOs:215, 216, 217, 275, 276 and 277, respectively; or
(20)SEQ ID NOs:218, 219, 220, 278, 279 and 280, respectively;
15 wherein the antibody or antigen-binding fragment thereof specifically binds to CD73,
preferably human CD73.

- [0075]** According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to one of SEQ
20 ID NOs:33, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 35, 37 or 39, or a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs:34, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 36, 38 or 40.
25 According to one preferred embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof of the invention comprises a heavy chain variable region having the polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:33, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 35, 37 or 39, and a light chain variable region having a
30 polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such

as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:34, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 36, 38 or 40, respectively.

[0076] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof of the invention, comprising:

- 5 (1) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:33, and a light chain variable region having the polypeptide sequence of SEQ ID NO:34;
- (2) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of
10 SEQ ID NO:2;
- (3) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;
- (4) a heavy chain variable region having the polypeptide sequence of SEQ ID
15 NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;
- (5) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;
- 20 (6) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;
- (7) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of
25 SEQ ID NO:12;
- (8) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;
- 30 (9) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;

- (10) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18;
- 5 (11) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20;
- (12) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21, and a light chain variable region having the polypeptide sequence of SEQ ID NO:22;
- 10 (13) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23, and a light chain variable region having the polypeptide sequence of SEQ ID NO:24;
- (14) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:25, and a light chain variable region having the polypeptide sequence of SEQ ID NO:26;
- 15 (15) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:27, and a light chain variable region having the polypeptide sequence of SEQ ID NO:28;
- (16) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:29, and a light chain variable region having the polypeptide sequence of SEQ ID NO:30;
- 20 (17) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:31, and a light chain variable region having the polypeptide sequence of SEQ ID NO:32;
- (18) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:35, and a light chain variable region having the polypeptide sequence of SEQ ID NO:36;
- 25 (19) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:37, and a light chain variable region having the polypeptide sequence of SEQ ID NO:38; or
- 30

(20) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:39, and a light chain variable region having the polypeptide sequence of SEQ ID NO:40.

5 [0077] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 41, 42, 43, 101, 102 and 103, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:1, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:2. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1; and a light chain variable region having the polypeptide sequence of SEQ ID NO:2.

15 [0078] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 44, 45, 46, 104, 105 and 106, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:3, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:4. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3; and a light chain variable region having the polypeptide sequence of SEQ ID NO:4.

25 [0079] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 47, 48, 49, 107, 108 and 109, respectively. In another embodiment, the isolated monoclonal antibody or

antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:5, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:6. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5; and a light chain variable region having the polypeptide sequence of SEQ ID NO:6.

[0080] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 50, 51, 52, 110, 111 and 112, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:7, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:8. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7; and a light chain variable region having the polypeptide sequence of SEQ ID NO:8.

[0081] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 53, 54, 55, 113, 114 and 115, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:9, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:10. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy

chain variable region having the polypeptide sequence of SEQ ID NO:9; and a light chain variable region having the polypeptide sequence of SEQ ID NO:10.

[0082] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 56, 57, 58, 116, 117 and 118, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:11, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:12. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11; and a light chain variable region having the polypeptide sequence of SEQ ID NO:12.

[0083] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 59, 60, 61, 119, 120 and 121, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:13, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:14. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13; and a light chain variable region having the polypeptide sequence of SEQ ID NO:14.

[0084] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 62, 63, 64, 122, 123 and 124, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a

polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:15, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:16. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15; and a light chain variable region having the polypeptide sequence of SEQ ID NO:16.

[0085] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 65, 66, 67, 125, 126 and 127, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:17, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:18. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17; and a light chain variable region having the polypeptide sequence of SEQ ID NO:18.

[0086] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 68, 69, 70, 128, 129 and 130, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:19, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:20. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19; and a light chain variable region having the polypeptide sequence of SEQ ID NO:20.

[0087] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 71, 72, 73, 131, 132 and 133, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:21, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:22. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21; and a light chain variable region having the polypeptide sequence of SEQ ID NO:22.

[0088] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 74, 75, 76, 134, 135 and 136, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:23, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:24. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23; and a light chain variable region having the polypeptide sequence of SEQ ID NO:24.

[0089] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 77, 78, 79, 137, 138 and 139, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:25, and a light chain variable

region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:26. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:25; and a light chain variable region having the polypeptide sequence of SEQ ID NO:26.

[0090] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 80, 81, 82, 140, 141 and 142, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:27, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:28. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:27; and a light chain variable region having the polypeptide sequence of SEQ ID NO:28.

[0091] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 83, 84, 85, 143, 144 and 145, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:29, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:30. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:29; and a light chain variable region having the polypeptide sequence of SEQ ID NO:30.

[0092] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1,

LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 86, 87, 88, 146, 147 and 148, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:31, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:32. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:31; and a light chain variable region having the polypeptide sequence of SEQ ID NO:32.

[0093] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 89, 90, 91, 149, 150 and 151, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:33, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:34. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:33; and a light chain variable region having the polypeptide sequence of SEQ ID NO:34.

[0094] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 92, 93, 94, 152, 153 and 154, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:35, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:36. Preferably,

the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:35; and a light chain variable region having the polypeptide sequence of SEQ ID NO:36.

[0095] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 95, 96, 97, 155, 156 and 157, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:37, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:38. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:37; and a light chain variable region having the polypeptide sequence of SEQ ID NO:38.

[0096] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 98, 99, 100, 158, 159 and 160, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:39, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:40. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:39; and a light chain variable region having the polypeptide sequence of SEQ ID NO:40.

[0097] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 161, 162, 163, 221, 222 and 223, respectively. In another embodiment, the isolated monoclonal

antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:1, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:2. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1; and a light chain variable region having the polypeptide sequence of SEQ ID NO:2.

[0098] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 164, 165, 166, 224, 225 and 226, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:3, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:4. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3; and a light chain variable region having the polypeptide sequence of SEQ ID NO:4.

[0099] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 167, 168, 169, 227, 228 and 229, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:5, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:6. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof

comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5; and a light chain variable region having the polypeptide sequence of SEQ ID NO:6.

[00100] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1,

5 LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 170, 171, 172, 230, 231 and 232, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:7, and a light chain
10 variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:8. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7; and a light chain variable region having the polypeptide sequence of SEQ ID NO:8.

15 **[00101]** In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 173, 174, 175, 233, 234 and 235, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region
20 having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:9, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:10. Preferably, the isolated monoclonal antibody or antigen-binding fragment
25 thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9; and a light chain variable region having the polypeptide sequence of SEQ ID NO:10.

[00102] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1,
30 LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 176, 177, 178, 236, 237 and 238, respectively. In another embodiment, the isolated monoclonal

- antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:11, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:12. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11; and a light chain variable region having the polypeptide sequence of SEQ ID NO:12.
- 10 **[00103]** In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 179, 180, 181, 239, 240 and 241, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region
- 15 having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:13, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:14. Preferably, the isolated monoclonal antibody or antigen-binding fragment
- 20 thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13; and a light chain variable region having the polypeptide sequence of SEQ ID NO:14.
- [00104]** In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1,
- 25 LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 182, 183, 184, 242, 243 and 244, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:15, and a light
- 30 chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID

NO:16. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15; and a light chain variable region having the polypeptide sequence of SEQ ID NO:16.

- 5 **[00105]** In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 185, 186, 187, 245, 246 and 247, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region
10 having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:17, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:18. Preferably, the isolated monoclonal antibody or antigen-binding fragment
15 thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17; and a light chain variable region having the polypeptide sequence of SEQ ID NO:18.

- [00106]** In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1,
20 LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 188, 189, 190, 248, 249 and 250, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:19, and a light
25 chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:20. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19; and a light chain variable region having the polypeptide sequence of SEQ ID
30 NO:20.

[00107] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 191, 192, 193, 251, 252 and 253, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:21, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:22. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21; and a light chain variable region having the polypeptide sequence of SEQ ID NO:22.

[00108] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 194, 195, 196, 254, 255 and 256, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:23, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:24. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23; and a light chain variable region having the polypeptide sequence of SEQ ID NO:24.

[00109] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 197, 198, 199, 257, 258 and 259, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region

having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:25, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID

5 NO:26. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:25; and a light chain variable region having the polypeptide sequence of SEQ ID NO:26.

[00110] In one embodiment, the invention relates to an isolated monoclonal antibody or
10 antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 200, 201, 202, 260, 261 and 262, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or
15 more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:27, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:28. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ
20 ID NO:27; and a light chain variable region having the polypeptide sequence of SEQ ID NO:28.

[00111] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 203, 204, 205,
25 263, 264 and 265, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:29, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more
30 preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:30. Preferably, the isolated monoclonal antibody or antigen-binding fragment

thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:29; and a light chain variable region having the polypeptide sequence of SEQ ID NO:30.

[00112] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 206, 207, 208, 266, 267 and 268, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:31, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:32. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:31; and a light chain variable region having the polypeptide sequence of SEQ ID NO:32.

[00113] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 209, 210, 211, 269, 270 and 271, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:33, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:34. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:33; and a light chain variable region having the polypeptide sequence of SEQ ID NO:34.

[00114] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1,

LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 212, 213, 214, 272, 273 and 274, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:35, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:36. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:35; and a light chain variable region having the polypeptide sequence of SEQ ID NO:36.

[00115] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 215, 216, 217, 275, 276 and 277, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:37, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:38. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:37; and a light chain variable region having the polypeptide sequence of SEQ ID NO:38.

[00116] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 218, 219, 220, 278, 279 and 280, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:39, and a light

chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:40. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:39; and a light chain variable region having the polypeptide sequence of SEQ ID NO:40.

[00117] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof of the invention, wherein the antibody or antigen-binding fragment thereof inhibits the enzyme activity of soluble and/or cell surface CD73. CD73 is a glycoposphatidylinositol (GPI)-anchored protein on the cell surface; it can also be shed to yield a catalytically active form in solution (Arias et al., J Cell Biol. 1997; 136(2):421-31). Here, the cell surface CD73 refers to the CD73 on the cell surface anchored by GPI; the soluble CD73 refers to those that are shed from the cell surface and catalytically active in the solution.

[00118] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof of the invention, wherein the antibody or antigen-binding fragment thereof prevents the dimerization of CD73. As used herein, “dimerization” refers to the formation of a CD73 dimer by two subunits. Preventing dimerization of CD73 refers to blocking or inhibiting the formation of a CD73 dimer.

[00119] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof of the invention, wherein the antibody or antigen-binding fragment thereof induces the internalization of CD73. As used herein, “internalization of CD73” refers to the movement of a CD73 protein from the surface of a cell to the inside areas of the cell.

[00120] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof of the invention, wherein the antibody or antigen-binding fragment thereof is capable of activating T cells by inhibiting the nucleotidase activity of CD73 and therefore reducing adenosine level.

[00121] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof of the invention, wherein the antibody or antigen-binding fragment thereof is chimeric.

[00122] According to another particular aspect, the invention relates to an isolated
5 monoclonal antibody or antigen-binding fragment thereof of the invention, wherein the antibody or antigen-binding fragment thereof is human or humanized.

[00123] According to another particular aspect, the invention relates to an isolated humanized monoclonal antibody or antigen-binding fragment thereof, wherein the isolated humanized antibody or antigen-binding fragment thereof comprises:

- 10 (1) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:286, and a light chain variable region having the polypeptide sequence of SEQ ID NO:293;
- (2) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:282, and a light chain variable region having the polypeptide sequence
15 of SEQ ID NO:290;
- (3) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:282, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;
- (4) a heavy chain variable region having the polypeptide sequence of SEQ ID
20 NO:282, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;
- (5) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:283, and a light chain variable region having the polypeptide sequence of SEQ ID NO:290;
- 25 (6) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:283, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;
- (7) a heavy chain variable region having the polypeptide sequence of SEQ ID
30 NO:283, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;

- (8) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:290;
- 5 (9) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;
- (10) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;
- 10 (11) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:285, and a light chain variable region having the polypeptide sequence of SEQ ID NO:290;
- (12) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:285, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;
- 15 (13) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:285, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;
- (14) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:283, and a light chain variable region having the polypeptide sequence of SEQ ID NO:293;
- 20 (15) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:293;
- 25 (16) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:285, and a light chain variable region having the polypeptide sequence of SEQ ID NO:293;
- (17) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:286, and a light chain variable region having the polypeptide sequence of SEQ ID NO:290;
- 30

- (18) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:286, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;
- 5 (19) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:286, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;
- (20) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:287, and a light chain variable region having the polypeptide sequence of SEQ ID NO:294;
- 10 (21) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:288, and a light chain variable region having the polypeptide sequence of SEQ ID NO:294;
- (22) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:289, and a light chain variable region having the polypeptide sequence of SEQ ID NO:294;
- 15 (23) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299;
- (24) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:295, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299;
- 20 (25) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:296, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299;
- 25 (26) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:297, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299; or
- (27) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:298, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299.
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[00124] In another general aspect, the invention relates to an isolated nucleic acid encoding a monoclonal antibody or antigen-binding fragment thereof of the invention. It will be appreciated by those skilled in the art that the coding sequence of a protein can be changed (e.g., replaced, deleted, inserted, etc.) without changing the amino acid sequence of the protein. Accordingly, it will be understood by those skilled in the art that nucleic acid sequences encoding monoclonal antibodies or antigen-binding fragments thereof of the invention can be altered without changing the amino acid sequences of the proteins.

[00125] In another general aspect, the invention relates to a vector comprising an isolated nucleic acid encoding a monoclonal antibody or antigen-binding fragment thereof of the invention. Any vector known to those skilled in the art in view of the present disclosure can be used, such as a plasmid, a cosmid, a phage vector or a viral vector. In some embodiments, the vector is a recombinant expression vector such as a plasmid. The vector can include any element to establish a conventional function of an expression vector, for example, a promoter, ribosome binding element, terminator, enhancer, selection marker, and origin of replication. The promoter can be a constitutive, inducible or repressible promoter. A number of expression vectors capable of delivering nucleic acids to a cell are known in the art and can be used herein for production of an antibody or antigen-binding fragment thereof in the cell. Conventional cloning techniques or artificial gene synthesis can be used to generate a recombinant expression vector according to embodiments of the invention.

[00126] In another general aspect, the invention relates to a host cell comprising an isolated nucleic acid encoding a monoclonal antibody or antigen-binding fragment thereof of the invention. Any host cell known to those skilled in the art in view of the present disclosure can be used for recombinant expression of antibodies or antigen-binding fragments thereof of the invention. In some embodiments, the host cells are E. coli TG1 or BL21 cells (for expression of, e.g., an scFv or Fab antibody), CHO-DG44 or CHO-K1 cells or HEK293 cells (for expression of, e.g., a full-length IgG antibody). According to particular embodiments, the recombinant expression vector is transformed into host cells by conventional methods such as chemical transfection, heat shock, or electroporation, where it is stably integrated into the host cell genome such that the recombinant nucleic acid is effectively expressed.

[00127] In another general aspect, the invention relates to a method of producing a monoclonal antibody or antigen-binding fragment thereof of the invention, comprising culturing a cell comprising a nucleic acid encoding the monoclonal antibody or antigen-binding fragment thereof under conditions to produce a monoclonal antibody or antigen-binding fragment thereof of the invention, and recovering the antibody or antigen-binding fragment thereof from the cell or cell culture (e.g., from the supernatant). Expressed antibodies or antigen-binding fragments thereof can be harvested from the cells and purified according to conventional techniques known in the art and as described herein.

[00128] Pharmaceutical Compositions

[00129] In another general aspect, the invention relates to a pharmaceutical composition, comprising an isolated monoclonal antibody or antigen-binding fragment thereof of the invention and a pharmaceutically acceptable carrier. The term “pharmaceutical composition” as used herein means a product comprising an antibody of the invention together with a pharmaceutically acceptable carrier. Antibodies of the invention and compositions comprising them are also useful in the manufacture of a medicament for therapeutic applications mentioned herein.

[00130] As used herein, the term “carrier” refers to any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, oil, lipid, lipid containing vesicle, microsphere, liposomal encapsulation, or other material well known in the art for use in pharmaceutical formulations. It will be understood that the characteristics of the carrier, excipient or diluent will depend on the route of administration for a particular application. As used herein, the term “pharmaceutically acceptable carrier” refers to a non-toxic material that does not interfere with the effectiveness of a composition according to the invention or the biological activity of a composition according to the invention. According to particular embodiments, in view of the present disclosure, any pharmaceutically acceptable carrier suitable for use in an antibody pharmaceutical composition can be used in the invention.

[00131] The formulation of pharmaceutically active ingredients with pharmaceutically acceptable carriers is known in the art, e.g., Remington: The Science and Practice of Pharmacy (e.g. 21st edition (2005), and any later editions). Non-limiting examples of additional ingredients include: buffers, diluents, solvents, tonicity regulating agents,

preservatives, stabilizers, and chelating agents. One or more pharmaceutically acceptable carrier can be used in formulating the pharmaceutical compositions of the invention.

[00132] In one embodiment of the invention, the pharmaceutical composition is a liquid formulation. A preferred example of a liquid formulation is an aqueous

5 formulation, i.e., a formulation comprising water. The liquid formulation can comprise a solution, a suspension, an emulsion, a microemulsion, a gel, and the like. An aqueous formulation typically comprises at least 50% w/w water, or at least 60%, 70%, 75%, 80%, 85%, 90%, or at least 95% w/w of water.

[00133] In one embodiment, the pharmaceutical composition can be formulated as an
10 injectable which can be injected, for example, via an injection device (e.g., a syringe or an infusion pump). The injection can be delivered subcutaneously, intramuscularly, intraperitoneally, intravitreally, or intravenously, for example.

[00134] In another embodiment, the pharmaceutical composition is a solid formulation, e.g., a freeze-dried or spray-dried composition, which can be used as is, or where to the
15 physician or the patient adds solvents, and/or diluents prior to use. Solid dosage forms can include tablets, such as compressed tablets, and/or coated tablets, and capsules (e.g., hard or soft gelatin capsules). The pharmaceutical composition can also be in the form of sachets, dragees, powders, granules, lozenges, or powders for reconstitution, for example.

[00135] The dosage forms can be immediate release, in which case they can comprise a
20 water-soluble or dispersible carrier, or they can be delayed release, sustained release, or modified release, in which case they can comprise water-insoluble polymers that regulate the rate of dissolution of the dosage form in the gastrointestinal tract or under the skin.

[00136] In other embodiments, the pharmaceutical composition can be delivered intranasally, intrabuccally, or sublingually.

25 **[00137]** The pH in an aqueous formulation can be between pH 3 and pH 10. In one embodiment of the invention, the pH of the formulation is from about 7.0 to about 9.5. In another embodiment of the invention, the pH of the formulation is from about 3.0 to about 7.0.

[00138] In another embodiment of the invention, the pharmaceutical composition
30 comprises a buffer. Non-limiting examples of buffers include: arginine, aspartic acid, bicine, citrate, disodium hydrogen phosphate, fumaric acid, glycine, glycyglycine,

histidine, lysine, maleic acid, malic acid, sodium acetate, sodium carbonate, sodium dihydrogen phosphate, sodium phosphate, succinate, tartaric acid, tricine, and tris(hydroxymethyl)-aminomethane, and mixtures thereof. The buffer can be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific buffers constitute alternative embodiments of the invention.

[00139] In another embodiment of the invention, the pharmaceutical composition comprises a preservative. Non-limiting examples of preservatives include: benzethonium chloride, benzoic acid, benzyl alcohol, bronopol, butyl 4-hydroxybenzoate, chlorobutanol, chlorocresol, chlorohexidine, chlorphenesin, o-cresol, m-cresol, p-cresol, ethyl 4-hydroxybenzoate, imidurea, methyl 4-hydroxybenzoate, phenol, 2-phenoxyethanol, 2-phenylethanol, propyl 4-hydroxybenzoate, sodium dehydroacetate, thiomerosal, and mixtures thereof. The preservative can be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific preservatives constitute alternative embodiments of the invention.

[00140] In another embodiment of the invention, the pharmaceutical composition comprises an isotonic agent. Non-limiting examples of the isotonic agents include a salt (such as sodium chloride), an amino acid (such as glycine, histidine, arginine, lysine, isoleucine, aspartic acid, tryptophan, and threonine), an alditol (such as glycerol, 1,2-propanediol propyleneglycol), 1,3-propanediol, and 1,3-butanediol), polyethyleneglycol (e.g. PEG400), and mixtures thereof. Another example of an isotonic agent includes a sugar. Non-limiting examples of sugars can be mono-, di-, or polysaccharides, or water-soluble glucans, including for example fructose, glucose, mannose, sorbose, xylose, maltose, lactose, sucrose, trehalose, dextran, pullulan, dextrin, cyclodextrin, alpha and beta-HPCD, soluble starch, hydroxyethyl starch, and sodium carboxymethylcellulose. Another example of an isotonic agent is a sugar alcohol, wherein the term "sugar alcohol" is defined as a C(4-8) hydrocarbon having at least one -OH group. Non-limiting examples of sugar alcohols include mannitol, sorbitol, inositol, galactitol, dulcitol, xylitol, and arabitol. The isotonic agent can be present individually or in the aggregate, in a

concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific isotonic agents constitute alternative embodiments of the invention.

[00141] In another embodiment of the invention, the pharmaceutical composition
5 comprises a chelating agent. Non-limiting examples of chelating agents include citric acid, aspartic acid, salts of ethylenediaminetetraacetic acid (EDTA), and mixtures thereof. The chelating agent can be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific chelating
10 agents constitute alternative embodiments of the invention.

[00142] In another embodiment of the invention, the pharmaceutical composition comprises a stabilizer. Non-limiting examples of stabilizers include one or more aggregation inhibitors, one or more oxidation inhibitors, one or more surfactants, and/or one or more protease inhibitors.

[00143] In another embodiment of the invention, the pharmaceutical composition comprises a stabilizer, wherein said stabilizer is carboxy-/hydroxycellulose and derivatives thereof (such as HPC, HPC-SL, HPC-L and HPMC), cyclodextrins, 2-methylthioethanol, polyethylene glycol (such as PEG 3350), polyvinyl alcohol (PVA), polyvinyl pyrrolidone, salts (such as sodium chloride), sulphur-containing substances such as monothioglycerol),
20 or thioglycolic acid. The stabilizer can be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific stabilizers constitute alternative embodiments of the invention.

[00144] In further embodiments of the invention, the pharmaceutical composition
25 comprises one or more surfactants, preferably a surfactant, at least one surfactant, or two different surfactants. The term “surfactant” refers to any molecules or ions that are comprised of a water-soluble (hydrophilic) part, and a fat-soluble (lipophilic) part. The surfactant can, for example, be selected from the group consisting of anionic surfactants, cationic surfactants, nonionic surfactants, and/or zwitterionic surfactants. The surfactant
30 can be present individually or in the aggregate, in a concentration from about 0.1 mg/ml

to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific surfactants constitute alternative embodiments of the invention.

[00145] In a further embodiment of the invention, the pharmaceutical composition comprises one or more protease inhibitors, such as, e.g., EDTA, and/or benzamidine hydrochloric acid (HCl). The protease inhibitor can be present individually or in the aggregate, in a concentration from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific protease inhibitors constitute alternative embodiments of the invention.

[00146] In another general aspect, the invention relates to a method of producing a pharmaceutical composition comprising a monoclonal antibody or antigen-binding fragment thereof of the invention, comprising combining a monoclonal antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

[00147] Methods of use

[00148] In another general aspect, the invention relates to a method of inhibiting the nucleotidase activity of CD73 in a subject in need thereof, the method comprising administering to the subject an isolated monoclonal antibody or antigen binding fragment thereof that specifically binds to CD73 or a pharmaceutical composition of the invention.

[00149] In another general aspect, the invention relates to a method of preventing the dimerization of CD73 in a subject in need thereof, the method comprising administering to the subject an isolated monoclonal antibody or antigen binding fragment thereof that specifically binds to CD73 or a pharmaceutical composition of the invention.

[00150] In another general aspect, the invention relates to a method of inducing the internalization of CD73 in a subject in need thereof, the method comprising administering to the subject an isolated monoclonal antibody or antigen binding fragment thereof that specifically binds to CD73 or a pharmaceutical composition of the invention. Internalization of the CD73 into the cell results in a reduction of the cell surface content of CD73. Levels of CD73 on the cell surface can be measured by methods known in the art, e.g., immunohistochemistry methods.

[00151] The functional activity of antibodies and antigen-binding fragments thereof that bind CD73 can be characterized by methods known in the art and as described herein.

Cell expressing CD73 can be preincubated with the antibody followed by measuring the CD73 nucleotidase activity on the cell surface in the presence of the antibody. Methods for characterizing antibodies and antigen-binding fragments thereof that bind CD73 include, but are not limited to, affinity and specificity assays including Biacore, ELISA, and OctetRed analysis; the functional activity of an anti-CD73 mAb can also be assessed in a nucleotidase activity assay. According to particular embodiments, the methods for characterizing antibodies and antigen-binding fragments thereof that bind CD73 include those described below.

[00152] In another general aspect, the invention relates to a method of treating a cancer in a subject in need thereof, comprising administering to the subject an isolated monoclonal antibody or antigen binding fragment thereof that specifically binds to CD73 or a pharmaceutical composition of the invention. The cancer can, for example, be selected from, but not limited to, a lung cancer, a gastric cancer, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

[00153] In another general aspect, the invention relates to a method of treating an inflammatory disease in a subject in need thereof, comprising administering to the subject an isolated monoclonal antibody or antigen binding fragment thereof that specifically binds to CD73 or a pharmaceutical composition of the invention.

[00154] According to embodiments of the invention, the pharmaceutical composition comprises a therapeutically effective amount of an anti-CD73 antibody or antigen-binding fragment thereof. As used herein, the term "therapeutically effective amount" refers to an amount of an active ingredient or component that elicits the desired biological or medicinal response in a subject. A therapeutically effective amount can be determined empirically and in a routine manner, in relation to the stated purpose.

[00155] As used herein with reference to anti-CD73 antibodies or antigen-binding fragments thereof, a therapeutically effective amount means an amount of the anti-CD73

antibody or antigen-binding fragment thereof that modulates a tumor reduction response in a subject in need thereof. Also, as used herein with reference to anti-CD73 antibodies or antigen-binding fragments thereof, a therapeutically effective amount means an amount of the anti-CD73 antibody or antigen-binding fragment thereof that results in
5 treatment of a disease, disorder, or condition; prevents or slows the progression of the disease, disorder, or condition; or reduces or completely alleviates symptoms associated with the disease, disorder, or condition.

[00156] According to particular embodiments, the disease, disorder or condition to be treated is cancer, preferably a cancer selected from the group consisting of a lung cancer,
10 a gastric cancer, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous
15 leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors. According to other particular embodiments, the disease, disorder or condition to be treated is an inflammatory disease.

[00157] According to particular embodiments, a therapeutically effective amount refers to the amount of therapy which is sufficient to achieve one, two, three, four, or more of
20 the following effects: (i) reduce or ameliorate the severity of the disease, disorder or condition to be treated or a symptom associated therewith; (ii) reduce the duration of the disease, disorder or condition to be treated, or a symptom associated therewith; (iii) prevent the progression of the disease, disorder or condition to be treated, or a symptom associated therewith; (iv) cause regression of the disease, disorder or condition to be
25 treated, or a symptom associated therewith; (v) prevent the development or onset of the disease, disorder or condition to be treated, or a symptom associated therewith; (vi) prevent the recurrence of the disease, disorder or condition to be treated, or a symptom associated therewith; (vii) reduce hospitalization of a subject having the disease, disorder or condition to be treated, or a symptom associated therewith; (viii) reduce
30 hospitalization length of a subject having the disease, disorder or condition to be treated, or a symptom associated therewith; (ix) increase the survival of a subject with the disease,

disorder or condition to be treated, or a symptom associated therewith; (xi) inhibit or reduce the disease, disorder or condition to be treated, or a symptom associated therewith in a subject; and/or (xii) enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

- 5 **[00158]** The therapeutically effective amount or dosage can vary according to various factors, such as the disease, disorder or condition to be treated, the means of administration, the target site, the physiological state of the subject (including, e.g., age, body weight, health), whether the subject is a human or an animal, other medications administered, and whether the treatment is prophylactic or therapeutic. Treatment
10 dosages are optimally titrated to optimize safety and efficacy.

[00159] According to particular embodiments, the compositions described herein are formulated to be suitable for the intended route of administration to a subject. For example, the compositions described herein can be formulated to be suitable for intravenous, subcutaneous, or intramuscular administration.

- 15 **[00160]** As used herein, the terms “treat,” “treating,” and “treatment” are all intended to refer to an amelioration or reversal of at least one measurable physical parameter related to a cancer and/or an inflammatory disease, disorder or condition, which is not necessarily discernible in the subject, but can be discernible in the subject. The terms “treat,” “treating,” and “treatment,” can also refer to causing regression, preventing the
20 progression, or at least slowing down the progression of the disease, disorder, or condition. In a particular embodiment, “treat,” “treating,” and “treatment” refer to an alleviation, prevention of the development or onset, or reduction in the duration of one or more symptoms associated with the disease, disorder, or condition, such as a tumor or more preferably a cancer. In a particular embodiment, “treat,” “treating,” and
25 “treatment” refer to prevention of the recurrence of the disease, disorder, or condition. In a particular embodiment, “treat,” “treating,” and “treatment” refer to an increase in the survival of a subject having the disease, disorder, or condition. In a particular embodiment, “treat,” “treating,” and “treatment” refer to elimination of the disease, disorder, or condition in the subject.

- 30 **[00161]** According to particular embodiments, a composition of the invention is used in the treatment of a cancer and/or an inflammatory disease, disorder or condition. For

cancer therapy, it can be used in combination with another treatment including, but not limited to, a chemotherapy, an anti-CD20 mAb, an anti-TIM-3 mAb, an anti-LAG-3 mAb, an anti-CD47 mAb, an anti-apelin mAb, an anti-claudin18.2 mAb, an anti-DLL3 mAb, an anti-FR α (folate receptor alpha) mAb, an anti-TIP-1 mAb, an anti-CTLA-4 antibody, an anti-PD-L1 antibody, an anti-PD-1 antibody, a PD-1/PD-L1 therapy, other immuno-oncology drugs, an antiangiogenic agent, a radiation therapy, an antibody-drug conjugate (ADC), a targeted therapy, or other anticancer drugs. Anti-CD73 antibodies can be used to construct bispecific antibodies with partner mAbs against PD-1, PD-L1, LAG3, TIM-3, CTLA-4, EGFR, HER-2, CD19, CD20, CD33, CD47, CD3, apelin, claudin18.2, DLL3, TIP-1, folate receptor alpha (FR α), and/or other tumor surface antigens to treat cancers/tumors that express both CD73 and the specific tumor associated antigen.

[00162] As used herein, the term “in combination,” in the context of the administration of two or more therapies to a subject, refers to the use of more than one therapy. The use of the term “in combination” does not restrict the order in which therapies are administered to a subject. For example, a first therapy (e.g., a composition described herein) can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy to a subject.

[00163] In another general aspect, the invention relates to a method of determining a level of CD73 in a subject. The methods comprise (a) obtaining a sample from the subject; (b) contacting the sample with a monoclonal antibody or antigen-binding fragment thereof of the invention; and (c) determining a level of CD73 in the subject.

[00164] In another general aspect, the invention relates to a method of determining the ecto-5'-nucleotidase activity of CD73 in a subject, wherein the enzyme activity can be fully inhibited by the monoclonal antibody or antigen-binding fragment thereof of the invention. The methods comprise (a) obtaining a sample from the subject; (b) contacting

the sample with an antibody or antigen-binding fragment thereof of the invention; and (c) determining the ecto-5'-nucleotidase activity of CD73 in the subject.

[00165] As used herein, "sample" refers to a biological sample isolated from a subject and can include, but is not limited to, whole blood, serum, plasma, blood cells,

5 endothelial cells, tissue biopsies (e.g., a cancer tissue), lymphatic fluid, ascites fluid, interstitial fluid, bone marrow, cerebrospinal fluid, saliva, mucous, sputum, sweat, urine, or any other secretion, excretion, or other bodily fluids. A "blood sample" refers to whole blood or any fraction thereof, including blood cells, serum, and plasma.

[00166] In certain embodiments, the level of CD73 in the subject can be determined

10 utilizing assays selected from, but not limited to, a Western blot assay, an ELISA assay, and/or immunohistochemistry (IHC). Relative protein levels can be determined by utilizing Western blot analysis and IHC, and absolute protein levels can be determined by utilizing an ELISA assay. When determining the relative levels of CD73, the levels of CD73 can be determined between at least two samples, e.g., between samples from the
15 same subject at different time points, between samples from different tissues in the same subject, and/or between samples from different subjects. Alternatively, when determining absolute levels of CD73, such as by an ELISA assay, the absolute level of CD73 in the sample can be determined by creating a standard for the ELISA assay prior to testing the sample. A person skilled in the art would understand which analytical
20 techniques to utilize to determine the level of CD73 in a sample from the subject utilizing the antibodies or antigen-binding fragments thereof of the invention.

[00167] In certain embodiments, the ecto-5'-nucleotidase activity of CD73 in the subject can be determined using assays known in the art, e.g., see Geoghegan et al., MAbs 8:454-67 (2016) and Hay et al., Oncoimmunology Aug. 5(8):e1208875 (2016). A

25 person skilled in the art would understand which analytical techniques to utilize to determine the ecto-5'-nucleotidase activity of CD73 in a sample from the subject.

[00168] Utilizing methods of determining a level of CD73 or a level of the ecto-5'-nucleotidase activity of CD73 in a sample from a subject can lead to the diagnosis of abnormal (elevated, reduced, or insufficient) CD73 levels and/or activities in a disease

30 and making appropriate therapeutic decisions. Such a disease can be selected from, but not limited to, a cancer and/or an inflammatory disease. Additionally, by monitoring the

levels and/or activity of CD73 in a subject, the risk of developing a disease as indicated above can be determined based on the knowledge of the level and/or activity of CD73 in a particular disease and/or during the progression of the particular disease.

5

EMBODIMENTS

[00169] The invention provides also the following non-limiting embodiments.

[00170] Embodiment 1 is an isolated monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of

10

a. SEQ ID NOs:89, 90, 91, 149, 150 and 151, respectively;

b. SEQ ID NOs:41, 42, 43, 101, 102 and 103, respectively;

c. SEQ ID NOs:44, 45, 46, 104, 105 and 106, respectively;

d. SEQ ID NOs:47, 48, 49, 107, 108 and 109, respectively;

15

e. SEQ ID NOs:50, 51, 52, 110, 111 and 112, respectively;

f. SEQ ID NOs:53, 54, 55, 113, 114 and 115, respectively;

g. SEQ ID NOs:56, 57, 58, 116, 117 and 118, respectively;

h. SEQ ID NOs:59, 60, 61, 119, 120 and 121, respectively;

i. SEQ ID NOs:62, 63, 64, 122, 123 and 124, respectively;

20

j. SEQ ID NOs:65, 66, 67, 125, 126 and 127, respectively;

k. SEQ ID NOs:68, 69, 70, 128, 129 and 130, respectively;

l. SEQ ID NOs:71, 72, 73, 131, 132 and 133, respectively;

m. SEQ ID NOs:74, 75, 76, 134, 135 and 136, respectively;

n. SEQ ID NOs:77, 78, 79, 137, 138 and 139, respectively;

25

o. SEQ ID NOs:80, 81, 82, 140, 141 and 142, respectively;

p. SEQ ID NOs:83, 84, 85, 143, 144 and 145, respectively;

q. SEQ ID NOs:86, 87, 88, 146, 147 and 148, respectively;

r. SEQ ID NOs:92, 93, 94, 152, 153 and 154, respectively;

s. SEQ ID NOs:95, 96, 97, 155, 156 and 157, respectively; or

30

t. SEQ ID NOs:98, 99, 100, 158, 159 and 160, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds to CD73, preferably human CD73.

[00171] Embodiment 2 is an isolated monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1),

5 HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of

- a. SEQ ID NOs:209, 210, 211, 269, 270 and 271, respectively;
- b. SEQ ID NOs:161, 162, 163, 221, 222 and 223, respectively;
- c. SEQ ID NOs:164, 165, 166, 224, 225 and 226, respectively;
- 10 d. SEQ ID NOs:167, 168, 169, 227, 228 and 229, respectively;
- e. SEQ ID NOs:170, 171, 172, 230, 231 and 232, respectively;
- f. SEQ ID NOs:173, 174, 175, 233, 234 and 235, respectively;
- g. SEQ ID NOs:176, 177, 178, 236, 237 and 238, respectively;
- h. SEQ ID NOs:179, 180, 181, 239, 240 and 241, respectively;
- 15 i. SEQ ID NOs:182, 183, 184, 242, 243 and 244, respectively;
- j. SEQ ID NOs:185, 186, 187, 245, 246 and 247, respectively;
- k. SEQ ID NOs:188, 189, 190, 248, 249 and 250, respectively;
- l. SEQ ID NOs:191, 192, 193, 251, 252 and 253, respectively;
- m. SEQ ID NOs:194, 195, 196, 254, 255 and 256, respectively;
- 20 n. SEQ ID NOs:197, 198, 199, 257, 258 and 259, respectively;
- o. SEQ ID NOs:200, 201, 202, 260, 261 and 262, respectively;
- p. SEQ ID NOs:203, 204, 205, 263, 264 and 265, respectively;
- q. SEQ ID NOs:206, 207, 208, 266, 267 and 268, respectively;
- r. SEQ ID NOs:212, 213, 214, 272, 273 and 274, respectively;
- 25 s. SEQ ID NOs:215, 216, 217, 275, 276 and 277, respectively; or
- t. SEQ ID NOs:218, 219, 220, 278, 279 and 280, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds to CD73, preferably human CD73.

[00172] Embodiment 3 is the isolated monoclonal antibody or antigen-binding

30 fragment of embodiment 1 or 2, comprising a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:33, 1, 3, 5, 7, 9, 11, 13, 15, 17,

19, 21, 23, 25, 27, 29, 31, 35, 37 or 39, or a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:34, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 36, 38 or 40.

[00173] Embodiment 4 is the isolated monoclonal antibody or antigen-binding

5 fragment of any one of embodiments 1-3, comprising

- (a) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:33, and a light chain variable region having the polypeptide sequence of SEQ ID NO:34;
- 10 (b) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;
- (c) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;
- 15 (d) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;
- (e) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of
- 20 SEQ ID NO:8;
- (f) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;
- (g) a heavy chain variable region having the polypeptide sequence of SEQ ID
- 25 NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;
- (h) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;

- (i) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;
- 5 (j) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18;
- (k) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20;
- 10 (l) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21, and a light chain variable region having the polypeptide sequence of SEQ ID NO:22;
- (m) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23, and a light chain variable region having the polypeptide sequence of SEQ ID NO:24;
- 15 (n) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:25, and a light chain variable region having the polypeptide sequence of SEQ ID NO:26;
- (o) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:27, and a light chain variable region having the polypeptide sequence of SEQ ID NO:28;
- 20 (p) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:29, and a light chain variable region having the polypeptide sequence of SEQ ID NO:30;
- (q) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:31, and a light chain variable region having the polypeptide sequence of SEQ ID NO:32;
- 25 (r) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:35, and a light chain variable region having the polypeptide sequence of SEQ ID NO:36;
- 30

(s) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:37, and a light chain variable region having the polypeptide sequence of SEQ ID NO:38; or

5 (t) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:39, and a light chain variable region having the polypeptide sequence of SEQ ID NO:40.

[00174] Embodiment 5 is the isolated monoclonal antibody or antigen-binding fragment thereof of any one of embodiments 1-4, wherein the monoclonal antibody or antigen-binding fragment thereof inhibits the enzyme activity of soluble and/or cell-
10 surface CD73.

[00175] Embodiment 6 is the isolated monoclonal antibody or antigen-binding fragment thereof of any one of embodiments 1-4, wherein the monoclonal antibody or antigen-binding fragment thereof prevents the dimerization of CD73.

[00176] Embodiment 7 is the isolated monoclonal antibody or antigen-binding
15 fragment thereof of any one of embodiments 1-4, wherein the monoclonal antibody or antigen-binding fragment thereof induces the internalization of CD73.

[00177] Embodiment 8 is the isolated monoclonal antibody or antigen-binding fragment of any one of embodiments 1-7, wherein the antibody or antigen-binding fragment thereof is chimeric.

20 **[00178]** Embodiment 9 is the isolated monoclonal antibody or antigen-binding fragment of any one of embodiments 1-8, wherein the antibody or antigen-binding fragment thereof is human or humanized.

[00179] Embodiment 10 is the isolated monoclonal antibody or antigen-binding
25 fragment thereof comprises:

(1) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:286, and a light chain variable region having the polypeptide sequence of SEQ ID NO:293;

30 (2) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:282, and a light chain variable region having the polypeptide sequence of SEQ ID NO:290;

- (3) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:282, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;
- 5 (4) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:282, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;
- (5) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:283, and a light chain variable region having the polypeptide sequence of SEQ ID NO:290;
- 10 (6) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:283, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;
- (7) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:283, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;
- 15 (8) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:290;
- (9) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;
- 20 (10) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;
- 25 (11) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:285, and a light chain variable region having the polypeptide sequence of SEQ ID NO:290;
- (12) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:285, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;
- 30

- (13) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:285, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;
- 5 (14) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:283, and a light chain variable region having the polypeptide sequence of SEQ ID NO:293;
- (15) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:293;
- 10 (16) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:285, and a light chain variable region having the polypeptide sequence of SEQ ID NO:293;
- (17) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:286, and a light chain variable region having the polypeptide sequence of
- 15 SEQ ID NO:290;
- (18) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:286, and a light chain variable region having the polypeptide sequence of SEQ ID NO:291;
- (19) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:286, and a light chain variable region having the polypeptide sequence of
- 20 SEQ ID NO:292;
- (20) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:287, and a light chain variable region having the polypeptide sequence of SEQ ID NO:294;
- 25 (21) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:288, and a light chain variable region having the polypeptide sequence of SEQ ID NO:294;
- (22) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:289, and a light chain variable region having the polypeptide sequence of
- 30 SEQ ID NO:294;

- (23) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:284, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299;
- 5 (24) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:295, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299;
- (25) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:296, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299;
- 10 (26) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:297, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299; or
- (27) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:298, and a light chain variable region having the polypeptide sequence of SEQ ID NO:299.
- 15
- [00180]** Embodiment 11 is the isolated monoclonal antibody or antigen-binding fragment of any one of embodiments 1-10, wherein the antibody or antigen-binding fragment thereof is capable of activating T cells.
- [00181]** Embodiment 12 is an isolated nucleic acid encoding the monoclonal antibody or antigen-binding fragment of any one of embodiments 1-11.
- 20 **[00182]** Embodiment 13 is a vector comprising the isolated nucleic acid of embodiment 12.
- [00183]** Embodiment 14 is a host cell comprising the vector of embodiment 13.
- [00184]** Embodiment 15 is a pharmaceutical composition, comprising the isolated monoclonal antibody or antigen-binding fragment of any one of embodiments 1-11 and a pharmaceutically acceptable carrier.
- 25 **[00185]** Embodiment 16 is a method of inhibiting the nucleotidase activity of CD73 in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 15.

[00186] Embodiment 17 is a method of preventing the dimerization of CD73 in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.

5 **[00187]** Embodiment 18 is a method of inducing the internalization of CD73 in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.

[00188] Embodiment 19 is a method of treating cancer in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 15.

10 **[00189]** Embodiment 20 is a method of producing the monoclonal antibody or antigen-binding fragment of any one of embodiments 1-11, comprising culturing a cell comprising a nucleic acid encoding the monoclonal antibody or antigen-binding fragment under conditions to produce the monoclonal antibody or antigen-binding fragment, and recovering the antibody or antigen-binding fragment from the cell or culture.

15 **[00190]** Embodiment 21 is a method of producing a pharmaceutical composition comprising the monoclonal antibody or antigen-binding fragment of any one of embodiments 1-11, comprising combining the monoclonal antibody or antigen-binding fragment with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

20 **[00191]** Embodiment 22 is a method of determining a level of CD73 in a subject, the method comprising:

- a. obtaining a sample from the subject;
- b. contacting the sample with the isolated monoclonal antibody or antigen-binding fragment thereof of any one of embodiments 1-11; and
- 25 c. determining a level of CD73 in the subject.

[00192] Embodiment 23 is the method of embodiment 22, wherein the sample is a tissue sample.

[00193] Embodiment 24 is the method of embodiment 23, wherein the tissue sample is a cancer tissue sample.

30 **[00194]** Embodiment 25 is the method of embodiment 22, wherein the sample is a blood sample.

[00195] Embodiment 26 is a method of determining the ecto-5'-nucleotidase activity of CD73 in a subject, the method comprising:

- a. obtaining a sample from the subject;
- b. contacting the sample with an isolated monoclonal antibody or antigen-binding fragment thereof of any one of embodiments 1-11; and
- c. determining the ecto-5'-nucleotidase activity of CD73 in the subject.

[00196] Embodiment 27 is the method of embodiment 26, wherein the sample is a tissue sample.

[00197] Embodiment 28 is the method of embodiment 27, wherein the tissue sample is a cancer tissue sample.

[00198] Embodiment 29 is the method of embodiment 26, wherein the sample is a blood sample.

EXAMPLES

[00199] Example 1: Identification of anti-CD73 monoclonal antibodies

[00200] Mice were immunized with a mixture of human CD73 (huCD73-HIS; containing residues 27-547) and mouse CD73 (mCD73-HIS; containing residues 29-549) fusion proteins, both with the HIS tag at the C-terminus. Plasma titer was determined by ELISA. After euthanization, spleens and lymph nodes were collected to produce hybridomas. Hybridomas were grown in 384-well tissue culture plates and supernatants from individual wells were screened by ELISA using huCD73-HIS and fluorescence activated cell sorting (FACS) using MDA-MB-231 cells. Positive clones were further analyzed by a nucleotidase activity assay with CD73 immobilized on the plate. Top positive clones that showed inhibition of the nucleotidase activity were isolated and sequenced.

[00201] Sequences of heavy and light chain variable regions for anti-CD73 monoclonal antibodies are provided in Tables 1 and 2, and the CDR regions for the anti-CD73 monoclonal antibodies are provided in Tables 3-6.

Table 1: Sequences of heavy chain variable regions for anti-CD73 mAbs

mAb clones	VH	ID
37C7A	QVTLKESGPGILQPSQTLSTLTCFSFGFSLSTFGMGVTWIRQPSGKGLEWLAHI WWDDDMYYNPALKSRLTISKDTSKNQVFLKIANVDTADTATYYCARSPITT	1

	VVADYWGQGSTLTVSS	
38C16A	QVQLQQSGAELMKPGASVKISCKATGYTFSSYWIEWVKQRPGHGLEWIGEIL PGSGSTNYNEKFKGKATFTADTSSNTAYMQLSSLTSEDSAVYYCARGDYFG SSYRGPYWQGGLTVTVSA	3
39G8A	QVQLQQPGAELVMPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGE IDPSGGYTNYNQKFKGKSTLTVDKSSSTAYMQLSRLTSEDSAVYYCARNYY YGSSGTM DYWGQGSTVTVSS	5
43E8A	QVQLQQSGAELMKPGASVKISCKATGYTFSNYWIEWVKQRPGHGLEWIGE LPGNVITNYNEKFKGKATFTADTSSNTAYMQLSSLTSEDSAVYYCARRGDD GYLYAMDYWGQGSTVTVSS	7
46J3A	EVQLQQSGPELVKPGASVKISCKASGYTFTDYYMHVVKQKPGQGLEWIGE YPGSGNTYYNEKFKGKATLTADRSSSTVYMLLSSLTSEDSAVYFCARYWDY YGSTYGYFDVWGAGTTVTVSS	9
46O19A	EVQLQQSGPELVKPGASVKMSCKASGYTFTDYNMHVVKQSHGKSLEWIGY INPNNGGTSYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARDYF WYFDVWGTGTTVTVSS	11
47H24A	QVQLKQSGPGLVQPSQSLTITCTVSGFSLTSYGVHWVRQSPGKGLEWLGVIW SGGSTDYNAAFISRLSISKDNSKSQVFFKMNSLQANDTAIYYCASQYVAYWG QGLTVTVST	13
49O5A	QVQLQQPGTELKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGN INPSNGGTNYNEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARSKSN YGYAMDYWGQGSTVTVSS	15
50B10A	QVQLQQPGAELVMPGASVKLSCKSSGYTFTSYWMHWVKQRPGQGLEWIGE NPSNGRTNYNEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARSEGRV YYDYFYAMDYWGQGSTVTVSS	17
50H17A	QVQLQQSGAELVRPGTSVKVSKASGYAFTNYLIEWVKQRPGQGLEWIGVI NPGSGGTNYNEKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARDYYW YFDVWGTGTTVTVSS	19
50E16A	ELARPWASVKISCQAFYTFRRMHFAIRDNTNYWMQWVKQRPGQGLEWIGAI YPGNGDTSYNQKFKVKATLTADKSSSTAYMQLSSLTSEDSAVYYCATYYSN YGGAMDYWGQGSTVTVSS	21
50F1A	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHVVKQAPGKGLKWMGW INTETGEPTYADDFKGRFAFSLETSASTAYLQINNLKNETATYFCARDIFWA MDYWGQGSTVTVSS	23
54I14A	QAYLQQSGAELVRPGASVKMSCKASGYTFTSYNMHWVKQTPRQGLEWIGA IYPGNGDTSYNQKFKGKATLTVDKSSSTAYMQLSSLTSEDSAVYFCARYDY DAMDYWGQGSTVTVSS	25
52K11A	EIQLQQSGAELVMPGASVKISCKASGYSFTGYNMNVVKQSHGKSLEWIGNIN PYYGSTSYNQKFKGKATLTVDKSSSTAYMQLNSLTSKDSAVYYCAGSSYVD YAMDYWGQGSTVTVSS	27
53H21A	EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGY IIPYNDGTKYNEKFEGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARWGNW DYFDYWGQGTTLTVSS	29
53I12A	QVQLQQPGAELVMPGASVKLSCKAPGYTFTSYWINVVKQRPGQGLEWIGNI YAGSSSSNYNEKFKSKATLTVDTSSTAYMQLSSLTSDDSAVYYCARSGHGY DGFAYWGQGLTVTVSA	31
60A9A	QVQLQQSGAELVMPGASVKISCKASGYAFSSYWMNVVKQRPGKGLEWIGQ IYPGDGDTYYSGKFKGKATLTAAKSSSTAYMQLSSLTSEDSAVYFCAREAIY YGNVFTYWGQGLTVTVSA	33
39J9A	QVQLQQSGAELVRPGTSVKVSKASGYAFTNYLIEWIKQRPGQGLEWIGLIN PGSGGTNYIEKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARRGDYYG NPFDYWGQGTTLTVSS	35
49H8A	QVQLQQPGAELVMPGASVKLSCKASGYTFTNYWMQWVRQRPGQGLEWIGE IDPSDNYTHYNQKFKGEATLTVDTSSTAYMQLSSLTSEDSAVYYCARGYY GYSPSWFAYWGQGLTVTVSA	37

51B10A	EVQLQQSGPELVKPGASVKISCKASGYSTGYMHVWKQSHVKSLEWIGRI NPYSGATNSNQNFKDKASLTVDKSSSTAYMELHSLTSEDSAVYYCARSYYG AMDYWGQGTSTVTVSS	39
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VH: heavy chain variable region

Table 2: Sequences of light chain variable regions for anti-CD73 mAbs

mAb	VL	ID
37C7A	DVVMQTQTPSLPVS LGDQASISCRSSQN LVHSYGNTYLHWY LQKPGQSPKLLIY KVS NRFS GVPDR FSGSGSGTDFTLKISRVEAEDLGVYFCSQNTHPWTFGGGTK LEIQ	2
38C16A	DVVMQTQTPSLSVTIGQPASISCKSSQSLLDS DGRTYLNWLLQRP GQSPKRLIYL VSKLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGVYVCWQGHFPHFTFGGGGTK LEIK	4
39G8A	DIVMTQSPSSLAMSVGQKVTMSCKSSQSLLNSSNQKNYLA WYQQKPGQSPKLL VYFASTRESGVPDRFIGSGSGTDFTLTISSVQAEDLADYFCQQHYSTPYTFGGGT KLEIK	6
43E8A	DIVLTQSPASLAVSLGQRATISCRASESDNYGISFMNW FQQKPGQPPKLLIYAA SNQGS GVPARFSGSGSGTDFTSLNIHPMEEDDTAM YFCQQSKEVPFTFGSGTKLE IK	8
46J3A	DVQITQSPSYLAASPGETITINCRASKNISKYLA WYQEIPGKTYNLLIYSGSTLQS GIPSRFSGSGSGTDFTLTISSELEDFAMYYCQQHNEYPFTFGSGTKLEIK	10
46O19A	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYQEKPGQPPKVL IYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSYPLTFGAG TKLELK	12
47H24A	DIQMTQSPSSLSASLGERVSLTCRASQDIGSRLTWLQQEPDGTIKRLIYATSSLDS GVPKRFSGSRSGSDYSLTISSELEDFVDYFCLQYASSPFTFGSGTKLEIK	14
49O5A	DIQMTQSSSYLSVSLGGRVTITCEASDHIDNWLAWYQQKPGNAPRLLISGATSL ETGVPSRFSGSGSGKDYTLTISLTQTEDVATYYCQQYWSSPFTFGSGTKLEIK	16
50B10A	DIQMTQTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKLLIYYSRL HSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNLTPWTFGGGTKLEIK	18
50H17A	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYQQKPGQPPKVL IYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSYPLTFGAG TKLELK	20
50E16A	DIVMTQFHKFMSTSVGDRVSITCKASQDVGTAVAWYQQKPGQSPKLLIYWAST RHTGVPDRFTGSGSGTDFTLTISNVQSEDADYFCQQYSSYPYTFGGGTKLEIK	22
50F1A	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLL IYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSYPLTFGAG TKLELK	24
54I14A	DIQMTQSPASLSASVGETVTITCRASENIYSYFAWYQQKQKSPQLLVYNAKTL AEGVPSRFSGSGSGTQFSLKINSLOPEDFGSYCQHGYGTPFTFGSGTKLEIK	26
52K11A	DIVMTQAAISNPVTLGTSASISCSNKSLLHSNDITYLYWYLQRP GQSPQLLIYR MSNLA SGVPDR FSGSGSGTDFTLRISRVEAEDVGVYYCAQMLERP WTFGGGTK LEIK	28
53H21A	QIVLTQSPALMASPGEKVTMTCSASSSVSYMYWYQQKPRSSPKPWLYLTSLN ASGVPARFSGSGSGTSYSLTISSEMAEDAATYYCQQWSSNPWTFGGGTKLEIK	30
53I12A	DIQMTQTSSLSASLGDRVTISCSASQGISNYLNWYQQKPDGTVKLLIYYTSSLH SGVPSRFSGSGSGTDYSLTISNLEPEDIATYYCQQYSKLPRTFGGGTKLEIK	32
60A9A	DIVMTQSPSSLAMSVGQKVTMSCKSSQSLLNSSNQKNYLA WYQQKPGQSPKLL VYFASTRD SGVPDR FIGGSGTDFTLTISSVQAEDLADYFCQQHYSTPLTFGAG TKLELK	34
39J9A	DIQMTQSPASLAASVGETVTITCRASENIYSLAWYQQKQKSPQLLIYNADTL EDGVPSRFSGSGSGTQYSMKINSMPEDTATYFCKQAYDVPLTFGAGTKLELK	36

49H8A	QIVLTQSPALMSASPGEKVTMTCSASSSVSYMYWYQQKPRSSPKPWYLTSLNLSGVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPPTFGSGTKLEIK	38
51B10A	DIKMTQSPSSMYASLGERVTITCKASQDINSYLSWFQQKPGKSPKTLIYRANRLVDGVPSRFSGSGSGQDYSLTISSLEYEDMGIYYCLQYDEFPLTFGAGTKLELK	40

VL: light chain variable region

Table 3: CDR regions 1-3 of heavy chain for anti-CD73 mAbs

mAb	HC CDR1	ID	HC CDR2	ID	HC CDR3	ID
37C7A	GFSLSTFGMG	41	IWWDDDM	42	ARSPITTVVADY	43
38C16A	GYTFSSYW	44	ILPGSGST	45	ARGDYFGSSYRGPY	46
39G8A	GYTFTSYW	47	IDPSGGYT	48	ARNYYYGSSGTMDY	49
43E8A	GYTFSNYW	50	ILPGNVIT	51	ARRGDDGYLYAMDY	52
46J3A	GYTFTDY	53	IYPGSGNT	54	ARYWDYYGSTYGYFDV	55
46O19A	GYTFTDYN	56	INPNNGGT	57	ARDYFWYFDV	58
47H24A	GFSLTSYG	59	IWSGGST	60	ASQYVAY	61
49O5A	GYTFTSYW	62	INPSNGGT	63	ARSKSNYGYAMDY	64
50B10A	GYTFTSYW	65	INPSNGRT	66	ARSEGRVYYDYFYAMDY	67
50H17A	GYAFTNYL	68	INPGSGGT	69	ARDYYWYFDV	70
50E16A	AIRDTNYW	71	IYPGNGDT	72	ATYYSNYGGAMDY	73
50F1A	GYTFTDYS	74	INTETGEP	75	ARDIFWAMDY	76
54I14A	GYTFTSYN	77	IYPGNGDT	78	ARYDYDAMDY	79
52K11A	GYSFTGYN	80	INPYYGST	81	AGSSYVDYAMDY	82
53H21A	GYTFTSYV	83	IIPYNDGT	84	ARWGNWDYFDY	85
53I12A	GYTFTSYW	86	IYAGSSSS	87	ARSGHGYDGFAY	88
60A9A	GYAFSSYW	89	IYPGDGDT	90	AREAIYYGNYVFTY	91
39J9A	GYAFTNYL	92	INPGSGGT	93	ARRGDYYGPNFDY	94
49H8A	GYTFTNYW	95	IDPSDNYT	96	ARGYYGYSPSWFAY	97
51B10A	GYSFTGYY	98	INPYSGAT	99	ARSYYGAMDY	100

HC: heavy chain; CDR: complementarity determining region; ID: SEQ ID NO

- 5 The HC CDRs for the anti-CD73 mAbs were determined utilizing the IMGT method (Lefranc, M.-P. et al., Nucleic Acids Res. 1999; 27:209-212).

Table 4: CDR regions 1-3 of light chain for anti-CD73 mAbs

mAb	LC CDR1	ID	LC CDR2	ID	LC CDR3	ID
37C7A	QNLVHSGNTY	101	KVS	102	SQNTHTVPWT	103
38C16A	QSLLDSDGRTY	104	LVS	105	WQGTHFPHT	106
39G8A	QSLNSSNQKNY	107	FAS	108	QQHYSTPYT	109
43E8A	ESVDNYGISF	110	AAS	111	QQSKEVPFT	112
46J3A	KNISKY	113	SGS	114	QQHNEYPFT	115
46O19A	QSLNNSGNQKNY	116	WAS	117	QNDYSYPLT	118
47H24A	QDIGSR	119	ATS	120	LQYASSPFT	121
49O5A	DHIDNW	122	GAT	123	QQYWSSPFT	124
50B10A	QDISNY	125	YTS	126	QQGNTLPWT	127
50H17A	QSLNNSGNQKNY	128	WAS	129	QNDYSYPLT	130

50E16A	QDVGTAVA	131	WAS	132	QQYSSYPYT	133
50F1A	QSLNSGNQKNY	134	WAS	135	QNDYSYPLT	136
54I14A	ENIYSY	137	NAK	138	QHHYGPFT	139
52K11A	KSLLSNDITY	140	RMS	141	AQMLERPWT	142
53H21A	SSVSY	143	LTS	144	QQWSSNPWT	145
53I12A	QGISNY	146	YTS	147	QQYSKLPRT	148
60A9A	QSLNSSNQKNY	149	FAS	150	QQHYSTPLT	151
39J9A	ENIYYS	152	NAD	153	KQAYDVPLT	154
49H8A	SSVSY	155	LTS	156	QQWSSNPPT	157
51B10A	QDINSY	158	RAN	159	LQYDEFPLT	160

LC: light chain; CDR: complementarity determining region

The LC CDRs for the anti-CD73 mAbs were determined utilizing the IMGT method (Lefranc, M.-P. et al., Nucleic Acids Res. 1999; 27:209-212).

5 Table 5: CDR regions 1-3 of heavy chain for anti-CD73 mAbs

mAb	HC CDR1	ID	HC CDR2	ID	HC CDR3	ID
37C7A	GFSLSTFGMGVT	161	HIWDDDDMYNPALKS	162	ARSPITTVVADY	163
38C16A	GYTFSSYWIE	164	EILPGSGSTNYNEKFKG	165	ARGDYFGSSYRGPY	166
39G8A	GYTFTSYWMH	167	EIDPSGGYTNYNQKFKG	168	ARNYYYGSSGTMDY	169
43E8A	GYTFSNYWIE	170	EILPGNVITNYNEKFKG	171	ARRGDDGYLYAMDY	172
46J3A	GYTFTDYMH	173	EIYPGSGNTYNEKFKG	174	ARYWDYYGSTYGYFDV	175
46O19A	GYTFTDYNMH	176	YINPNNGGTSYNQKFKG	177	ARDYFWYFDV	178
47H24A	GFSLTSYGVH	179	VIWSGGSTDYNAAFISR	180	ASQYVAY	181
49O5A	GYTFTSYWMH	182	NINPSNGGTNYNEKFKS	183	ARSKSNYGYAMDY	184
50B10A	GYTFTSYWMH	185	EINPSNGRTNYNEKFKS	186	ARSEGRVYYDYFYAMDY	187
50H17A	GYAFTNYLIE	188	VINPGSGGTNYNEKFKG	189	ARDYYWYFDV	190
50E16A	AIRDNYWMQ	191	AIYPGNGDTSYNQKFKV	192	ATYYSNYGGAMDY	193
50F1A	GYTFTDYSMH	194	WINTETGEPTYADDFKG	195	ARDIFWAMDY	196
54I14A	GYTFTSYNMH	197	AIYPGNGDTSYNQKFKG	198	ARYDYDAMDY	199
52K11A	GYSFTGYNMN	200	NINPYYGSTSYNQKFKG	201	AGSSYVDYAMDY	202
53H21A	GYTFTSYVMH	203	YIIPYNDGTYNEKFEG	204	ARWGNWDYFDY	205
53I12A	GYTFTSYWIN	206	NIYAGSSSSNYNEKFKS	207	ARSGHGYDGFAY	208
60A9A	GYAFSSYWMN	209	QIYPGDGDYYSYGKFKG	210	AREAIYYGNYVFTY	211
39J9A	GYAFTNYLIE	212	LINPGSGGTNYIEKFKG	213	ARRGDYYGNPFYD	214
49H8A	GYTFTNYWMQ	215	EIDPSDNYTHYNQKFKG	216	ARGYYGYSPSWFAY	217
51B10A	GYSFTGYMH	218	RINPYSGATNSNQNFKD	219	ARSYYGAMDY	220

HC: heavy chain; CDR: complementarity determining region

The HC CDRs for the anti-CD73 mAbs were determined utilizing a combination of IMGT (Lefranc, M.-P. et al., Nucleic Acids Res. 1999; 27:209-212) and Kabat (Elvin A. Kabat et al., Sequences of Proteins of Immunological Interest 5th ed. (1991)) methods.

Table 6: CDR regions 1-3 of light chain for anti-CD73 mAbs

mAb	LC CDR1	ID	LC CDR2	ID	LC CDR3	ID
37C7A	RSSQNLVHSYGNTYLH	221	KVSNRFS	222	SQNTHPVWT	223
38C16A	KSSQSLLDSDGRTYLN	224	LVSKLDS	225	WQGTDFPHT	226
39G8A	KSSQSLLNSSNQKNYLA	227	FASTRES	228	QQHYSTPYT	229
43E8A	RASESVDNYSIFMN	230	AASNQGS	231	QQSKEVPFT	232
46J3A	RASKNISKYLA	233	SGSTLQS	234	QQHNEYPFT	235
46O19A	KSSQSLLNSGNQKNYLT	236	WASTRES	237	QNDYSYPLT	238
47H24A	RASQDIGSRLT	239	ATSSLDS	240	LQYASSPFT	241
49O5A	EASDHIDNWL A	242	GATSLET	243	QQYWSSPFT	244
50B10A	RASQDISNYLN	245	YTSRLHS	246	QQGNTLPWT	247
50H17A	KSSQSLLNSGNQKNYLT	248	WASTRES	249	QNDYSYPLT	250
50E16A	KASQDVGTAVA	251	WASTRHT	252	QQYSSYPYT	253
50F1A	KSSQSLLNSGNQKNYLT	254	WASTRES	255	QNDYSYPLT	256
54I14A	RASENIYSYFA	257	NAKTLAE	258	QHHYGTPT	259
52K11A	SSNKSLLHSNDITYLY	260	RMSNLAS	261	AQMLERPWT	262
53H21A	SASSSVSYMY	263	LTSNLAS	264	QQWSSNPWT	265
53I12A	SASQGISNYLN	266	YTSSLHS	267	QQYSKLPRT	268
60A9A	KSSQSLLNSSNQKNYLA	269	FASTRDS	270	QQHYSTPLT	271
39J9A	RASENIYYSLA	272	NADTLED	273	KQAYDVPLT	274
49H8A	SASSSVSYMY	275	LTSNLAS	276	QQWSSNPPT	277
51B10A	KASQDINSYLS	278	RANRLVD	279	LQYDEFPLT	280

LC: light chain; CDR: complementarity determining region

The LC CDRs for the anti-CD73 mAbs were determined utilizing a combination of IMGT (Lefranc, M.-P. et al., Nucleic Acids Res. 1999; 27:209-212) and Kabat (Elvin A.

5 Kabat et al., Sequences of Proteins of Immunological Interest 5th ed. (1991)) methods.

[00202] Example 2: Production and purification of mAbs from culture media of transfected 293E cells

[00203] To obtain the recombinant anti-CD73 chimeric mAbs, the expression vectors containing the mouse variable regions (VH and VL) fused to the constant regions of human IgG1 heavy chain with LALA mutations (L234A/L235A) and kappa light chain, respectively, were transiently transfected into 293E cells. The recombinant antibodies produced in the suspension of the 293E cells were purified using Protein A affinity chromatography.

15 [00204] Example 3: Inhibition of the nucleotidase activity of immobilized CD73 by purified anti-CD73 chimeric mAbs

[00205] HIS-tagged human CD73 (huCD73-HIS, 60 μ L/well at 0.15 μ g/mL) in assay buffer (25 mM Tris-HCl, pH7.5, and 5 mM MgCl₂) was coated on nickel coated plates

(ThermoFisher Scientific, Cat#: 15242) at 4°C overnight. After washing, anti-CD73 chimeric mAbs were added and incubated at room temperature for 1 hour. The enzymatic reaction measuring the nucleotidase activity of CD73 was initiated by adding 100 µM AMP to the plate. The reaction proceeded for 20 min at 37°C. The free phosphate was detected and quantified by Malachite Green Phosphate Detection Kit (R&D Systems, Cat#: DY996). Inhibition of the nucleotidase activity of immobilized CD73 by anti-CD73 chimeric mAbs is shown in FIGs. 1A and 1B. No Antibody, enzyme reaction with no inhibitor; AMPCP (adenosine 5'(α , β -methylene)diphosphate) was used as a control for inhibition of enzyme activity.

10 **[00206] Example 4: ELISA binding assay with chimeric mAbs**

[00207] HIS-tagged human CD73 (huCD73-HIS, 50 µL/well at 5 µg/mL (85 nM)) (BPS Bioscience, Cat#: 71184) in carbonate coating buffer was coated on an ELISA plate at 4°C overnight. After washing by TBST buffer (TBS buffer with 0.05% Tween 20), the ELISA plate was blocked by 5% BSA in TBST at room temperature for 1 hour and washed again. Chimeric anti-CD73 antibodies were added, mixed and incubated for 1 hour at room temperature. The plate was washed and the binding of anti-CD73 antibodies to the immobilized huCD73-HIS was detected by adding anti-human IgG conjugated to horseradish peroxidase (anti-hIgG-HRP) (ThermoFisher Scientific, Cat#: H10007) and incubating for 1 hour. The plate was washed, and the ELISA was developed using One-step Detection Solution (ThermoFisher Scientific, Cat#: 34029) and measured as the absorbance at 450 nm. The results for the binding of anti-CD73 mAbs to CD73 are shown in FIGs. 2A-2E.

[00208] Example 5: Inhibition of the nucleotidase activity of soluble CD73 by anti-CD73 mAbs

25 **[00209]** This assay was carried out using recombinant human CD73 in solution rather than immobilized on a plate. HIS-tagged human CD73 (huCD73-HIS) (BPS Bioscience, Cat#: 71184) at final concentration of 0.5 nM and various concentrations of anti-CD73 antibodies were incubated in assay buffer (25 mM Tris-HCl, pH7.5, 5 mM MgCl₂, 140 mM NaCl, and 0.1% BSA) for 20 minutes at 37°C. The enzymatic reaction was initiated by adding AMP (Sigma Aldrich, Cat#: A2252) to the final concentration of 400 µM. After incubating the plate at 37°C for 20 min, the concentration of inorganic phosphate

was determined by Malachite Green Phosphate Detection Kit (R&D Systems, Cat#: DY996). Two chimeric anti-CD73 mAbs 60A9A/IgG4 and 39G8A/IgG4 were made by fusing the VH and VL regions of mouse mAbs 60A9A and 39G8A to the constant regions of human IgG4 heavy chain and kappa light chain, respectively. The chimeric antibodies were expressed in CHO cells and purified using Protein A affinity chromatography and tested in this assay. The results for the inhibition of the nucleotidase activity of soluble CD73 by 60A9A/IgG4 and 39G8A/IgG4 are shown in FIG. 3.

[00210] Example 6: Humanization of anti-CD73 mAbs

[00211] The mouse anti-CD73 mAbs 60A9A and 39G8A were humanized to reduce the potential of immunogenicity when used in human patients. The sequences of the variable regions of the heavy and light chains (VH and VL) were compared with the human antibody sequences in the Protein Data Bank (PDB) database and homology models were built. The CDRs in both the heavy and light chains of the mouse mAbs were grafted into human frameworks that have the highest possibility of maintaining the proper structure likely required for antigen binding. The sequences of the humanized VH and VL regions are shown in Tables 7 and 8.

[00212] The humanized VH and VL regions were fused to the constant regions of human IgG1 heavy chain with LALA mutations (L234A/L235A) and kappa light chain, respectively. Constructs corresponding to the mAb sequences were used for transient transfection in 293E cells and the humanized mAbs were purified using Protein A chromatography. The humanized mAbs were tested in an enzyme activity assay with soluble human CD73. In this assay, 0.4 nM huCD73 was incubated with the mAbs for 1 hour on ice before the enzyme reaction was initiated by adding AMP to a final concentration of 100 μ M. The IC₅₀ values for the humanized mAbs are shown in Table 9.

[00213] Humanized mAb 60A9-H5L4 on human IgG4/kappa backbone (60A9-H5L4/IgG4) was analyzed in the ELISA binding assay; the IgG4 chimeric version of 60A9A (60A9A/IgG4) was used as control. The ELISA binding result is shown in FIG. 4A. 60A9-H5L4/IgG4 was also analyzed for its ability to inhibit the nucleotidase activity of soluble CD73 under the conditions in Example 5. The result is shown in FIG. 4B.

Table 7: Sequences of humanized heavy chain variable regions for anti-CD73 mAbs

VH	Sequence	ID
60A9-H1	EVQLVESGGGLVQPGQSLKLSCASGYAFSSYWMNWVRQAPGKGLEWMG QIYPGDGDTYYNPSVKGRFTISADTSKNTAYLQLNNLRAEDTAVYYCAREAI YYGNYVFTYWGQGTLLTVSS	282
60A9-H2	EVQLVESGGGLVQPGGSLRLSCKASGYAFSSYWMNWVRQAPGKGLEWVG QIYPGDGDTYYNPSVKGRFTISADTSKNTLYLQMNSLRAEDTAVYYCAREAI YYGNYVFTYWGQGTLLTVSS	283
60A9-H3	EVQLVESGGGLVQPGQSLKLSCASGYAFSSYWMNWVRQAPGKGLEWIGQ IYPGDGDTYYNPSVKGRATLSADKSKNTAYLQLNNLRAEDTAVYYCAREAI YYGNYVFTYWGQGTLLTVSS	284
60A9-H4	EVQLVESGGGLVQPGQSLKLSCASGYAFSSYWMNWVRQAPGKGLEWIGQ IYPGDGDTYYSGSVKGRATLSADKSKNTAYLQLNNLRAEDTAVYYCAREAI YYGNYVFTYWGQGTLLTVSS	285
60A9-H5	EVQLVESGGGLVQPGQSLKLSCASGYAFSSYWMNWVRQAPGKGLEWIGQ IYPGDGDTYYSGKFKGRATLSADKSKNTAYLQLNNLRAEDTAVYYCAREAI YYGNYVFTYWGQGTLLTVSS	286
60A9-H6	QVQLVQSGAEVKKPGSSVTVSCKASGYAFSSYWMNWVRQAPGRGLEWIGQ IYPGDGDTYYAPRFQGRATLTADKSTSTAYLELNSLRPEDTAVYFCAREAIY YGNYVFTYWGQGTLLTVSS	295
60A9-H7	QVQLVQSGAEVKKPGSSVTVSCKASGYAFSSYWMNWVRQAPGRGLEWIGQ IYPGDGDTYYAPKFQGRATLTADKSTSTAYMELSSLRSEDATVYFCAREAIY YGNYVFTYWGQGTLLTVSS	296
60A9-H8	QVQLVQSGAEVKKPGSSVTVSCKASGYAFSSYWMNWVRQAPGRGLEWIGQ IYPGDGDTYYSGKFQGRATLTADKSTSTAYMELSSLRSEDATVYFCAREAIY YGNYVFTYWGQGTLLTVSS	297
60A9-H9	QVQLVQSGAEVKKPGSSVTVSCKASGYAFSSYWMNWVRQAPGRGLEWIGQ IYPGDGDTYYSGKFKGRATLSADKSKNTAYLQLNNLRAEDTAVYYCAREAI YYGNYVFTYWGQGTLLTVSS	298
39G8-H1	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGGGLEWIGE IDPSGGYTNYAQKFQGRSTLTVDKSISTAYMELSRLRSDDTAVYYCARNYYY GSSGTMDYWGQGTLLTVSS	287
39G8-H2	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGGGLEWIGE IDPSGGYTNYAQKFQGRSTLTVDTSISTAYMELSRLRSDDTAVYYCARNYYY GSSGTMDYWGQGTLLTVSS	288
39G8-H3	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGGGLEWIGE IDPSGGYTNYNQKFQGRSTLTVDKSISTAYMELSRLRSDDTAVYYCARNYYY GSSGTMDYWGQGTLLTVSS	289

Table 8: Sequences of humanized light chain variable regions for anti-CD73 mAbs

VL	Sequence	ID
60A9-L1	DIQMTQSPSLLSASLGDRVITITCKSSQSLNSSNQKNYLAWYQQKPGQSPKLL LIYFASTRDSGVPDRFSGSGSGTDFTLTISSELEPEDFATYYCQQHYSTPLTFGG GTKLEIK	290
60A9-L2	DIVMTQSPSLLSASLGDRVITITCKSSQSLNSSNQKNYLAWYQQKPGQSPKLL IYFASTRDSGVPDRFSGSGSGTDFTLTISSELEPEDFATYYCQQHYSTPLTFGGG TKLEIK	291
60A9-L3	DIQMTQSPSLLSASLGDRVITITCKSSQSLNSSNQKNYLAWYQQKPGQSPKLL LVYFASTRDSGVPDRFSGSGSGTDFTLTISSELEPEDFATYYCQQHYSTPLTFGG GTKLEIK	292
60A9-L4	DIVMTQSPSLLSASLGDRVITISCKSSQSLNSSNQKNYLAWYQQKPGQSPKLL VYFASTRDSGVPDRFSGSGSGTDFTLTISSELEPEDFATYFCQQHYSTPLTFGAG TKLEIK	293

60A9-L5	EIVMTQSPGTQSLSPGERATLSCKSSQSLNSSNQKNYLAWYQQRPGQAPRL LVYFASTRD SGVADRFSGSGSGTDFTLTISRLEPEDFAVYFCQQHYSTPLTFG QGTKVEVK	299
39G8-L3	DIVMTQSPDSLAVSLGERATMSCKSSQSLNSSNQKNYLAWYQQKPGQPPK LLVYFASTRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYFCQQHYSTPYTF GGGTKVEIK	294

Table 9: IC50 values for humanized mAbs in huCD73 enzymatic assay in solution

Name	IC50 (nM)
60A9-H1L1	3.34
60A9-H1L2	6.17
60A9-H1L3	4.96
60A9-H2L1	4.13
60A9-H2L2	11.20
60A9-H2L3	4.57
60A9-H3L1	2.94
60A9-H3L2	4.97
60A9-H3L3	2.09
60A9-H4L1	3.10
60A9-H4L2	4.97
60A9-H4L3	2.74

The name 60A9-H1L1 refers to the mAb constructed using VH 60A9-H1 and VL 60A9-L1. All the other humanized mAbs adopt the same naming rule.

5

[00214] Example 7: Inhibition of the nucleotidase activity of cell surface CD73 by anti-CD73 mAbs

[00215] A375 cells were harvested with PBS-EDTA (2 mM EDTA in PBS) and washed twice in PBS buffer supplemented with 0.1% BSA. 100,000 cells were plated in the presence of various concentrations of anti-CD73 antibodies and incubated for 20 min at 37°C. AMP was then added to the final concentration of 125 µM (Sigma Aldrich, Cat#: A2252) in a final volume of 60 µL and the reaction was kept at 37°C for 60 min. Plates were then centrifuged and 40 µL of supernatant was transferred to a new plate. ATP (Sigma Aldrich, Cat#: A9187) was added to make the final concentration of 100 µM in a final volume of 50 µL and the mixture was incubated at 37°C for 15 min. CellTiter-Glo® 2.0 reagent (Promega, Cat#: G9242) was added in a 1:1 ratio to determine the AMP level in the mixture and the enzyme activity in a given reaction was calculated based on the AMP concentration at the end of reaction. The results for the inhibition of CD73 activity

by the humanized anti-CD73 mAbs (on human IgG1 LALA/kappa backbone) in the cell-based assay are shown in Table 10.

[00216] Humanized anti-CD73 mAb 60A9-H5L4/IgG4, 60A9-H3L5/IgG4, 60A9-H5L5/IgG4, 60A9-H6L5/IgG4, 60A9-H7L5/IgG4, 60A9-H8L5/IgG4 and 60A9-H9L5/IgG4 were also tested in a cell-based assay using a phosphate detection method. In this assay, 50,000 A375 cells were washed and plated in assay buffer (25 mM Tris-HCl, pH7.5, 5 mM MgCl₂, 140 mM NaCl, and 0.1% BSA) and the enzyme reaction with a final AMP concentration of 400 μ M lasted 20 min at 37°C. Free phosphate produced by the hydrolysis of AMP was quantified using Malachite Green Phosphate Detection Kit (R&D Systems, Cat#: DY996). The assay results are shown in FIGs. 4C and 4D.

Table 10: IC50 values for humanized mAbs in cell-based CD73 activity assay

Name	IC50 (nM)
60A9-H2L4	46.03
60A9-H3L4	133.67
60A9-H3L3	65.65
60A9-H4L4	134.67
60A9-H5L1	61.02
60A9-H5L2	57.31
60A9-H5L3	36.41
60A9-H5L4	30.12
39G8-H1L3	49.62
39G8-H2L3	66.33
39G8-H3L3	56.12

[00217] Example 8: Inhibition of the nucleotidase activity of CD73 in patient serum samples by anti-CD73 mAbs

[00218] Serum samples from patients with colorectal cancer were used to assess the inhibitory activity of the humanized anti-CD73 mAb 60A9-H5L4/IgG4. 20 μ L of serum and 10 μ L of the anti-CD73 mAb in PBS buffer supplemented with 0.1% BSA were incubated at 37°C for 30 min. 10 μ L of 500 μ M AMP (Sigma Aldrich, Cat#: A2252) was added and the reaction mixture was incubated at 37°C for 30 min. ATP (Sigma Aldrich, Cat#: A9187) was then added to the final concentration of 100 μ M. 25 μ L of the reaction mixture was transferred to a white half-area 96-well plate. CellTiter-Glo® 2.0

reagent (Promega, Cat#: G9242) was added in a 1:1 ratio to the mixture and the residual AMP level was determined by measuring its inhibitory effect on ATP detection. Samples containing 100 μ M AMP and 100 μ M ATP were used to establish assay background and samples containing only 100 μ M ATP were used as positive control.

- 5 **[00219]** The inhibition of the nucleotidase activity of CD73 in patient serum samples by humanized anti-CD73 mAb 60A9-H5L4/IgG4 was analyzed at 1000 nM. The results are shown in FIG. 5.

[00220] Example 9: Activation of T cell proliferation by humanized anti-CD73 mAb 60A9-H5L4/IgG4

- 10 **[00221]** Primary human CD4⁺ T cells were isolated from frozen peripheral blood mononuclear cells using CD4⁺ T cell isolation kit (Miltenyi Biotec, Cat#: 130-096-533). Isolated CD4⁺ T cells at a density of 1,000,000 cells per mL in DPBS were labeled using CellTrace™ CFSE cell proliferation kit (Invitrogen, Cat#: C34554) at 37°C for 30 min. Cells were washed three times with cold DPBS and resuspended in AIM V media (Gibco, Cat#: 12055083). 100,000 cells per well were pre-incubated with various concentrations of 60A9-H5L4/IgG4 for 1 hour at 37°C. T cells were activated by the addition of T cell activation reagents, a combination of Dynabeads human T-activator CD3/CD28 (Gibco, Cat#: 11131D) at 1:1 bead-to-cell ratio and human IL-2 to the final concentration of 60 IU/mL. Thereafter, AMP (Sigma Aldrich, Cat#: A2252) was added to the final
- 15 concentration of 100 μ M. After 72 hours of incubation at 37°C, cells were stained with PE/Cy7 anti-human CD4 antibody (BioLegend, Cat#: 357409). Both PE/Cy7 and CFSE signals were analyzed on a Attune NxT flow cytometer. For the assay window, CFSE CD4⁺ cells in the absence of the T cell activation reagents had undergone no cell divisions and therefore were used to define the baseline while activated CFSE CD4⁺ cells
- 20 (treated with the T cell activation reagents) in the absence of AMP had undergone uninhibited cell division and therefore were used to define the maximum proliferation. The effect of 60A9-H5L4/IgG4 on the activation of T cell proliferation is shown in FIG. 6. The cells treated with AMP were suppressed as expected, presumably due to the production of adenosine by cell surface CD73, and the addition of the anti-CD73 mAb
- 25 60A9-H5L4/IgG4 at 75 nM activated the cells, suggesting the mAb has functional activity in T cell activation.
- 30

[00222] It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular
5 embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the present description.