

CLAIMS

It is claimed:

1. 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:
 - 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;
 - 10 d. SEQ ID NOs:47, 48, 49, 107, 108 and 109, respectively;
 - 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;
 - 15 i. SEQ ID NOs:62, 63, 64, 122, 123 and 124, respectively;
 - 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;
 - 20 n. SEQ ID NOs:77, 78, 79, 137, 138 and 139, respectively;
 - 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;
 - 25 s. SEQ ID NOs:95, 96, 97, 155, 156 and 157, respectively; or
 - 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.
2. An isolated monoclonal antibody or antigen-binding fragment thereof comprising
 - 30 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:

- a. SEQ ID NOs:209, 210, 211, 269, 270 and 271, respectively;
- b. SEQ ID NOs:161, 162, 163, 221, 222 and 223, respectively;
- 5 c. SEQ ID NOs:164, 165, 166, 224, 225 and 226, respectively;
- 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;
- 10 h. SEQ ID NOs:179, 180, 181, 239, 240 and 241, respectively;
- 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;
- 15 m. SEQ ID NOs:194, 195, 196, 254, 255 and 256, respectively;
- 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;
- 20 r. SEQ ID NOs:212, 213, 214, 272, 273 and 274, respectively;
- 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.

- 25 3. The isolated monoclonal antibody or antigen-binding fragment thereof of claim 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
- 30 or 40.

4. The isolated monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-3, comprising:

- 5 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;
- 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;
- 10 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;
- 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;
- 15 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;
- 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;
- 20 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;
- 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;
- 25 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;

- 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;
- 5 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;
- 10 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;
- 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;
- 15 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;
- 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;
- 20 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;
- 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
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- 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.
5. The isolated monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-4, wherein the monoclonal antibody or antigen-binding fragment thereof inhibits the enzyme activity of soluble and/or cell-surface CD73.
6. 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.
- 10 7. 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 induces the internalization of CD73.
8. The isolated monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-7, wherein the antibody or antigen-binding fragment thereof is chimeric.
- 15 9. The isolated monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-8, wherein the antibody or antigen-binding fragment thereof is human or humanized.
10. The isolated monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-9, wherein the antibody or antigen-binding fragment thereof comprises:
- 20 (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 of SEQ ID NO:290;
- 25 (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 NO:282, and a light chain variable region having the polypeptide sequence of SEQ ID NO:292;
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- (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;
- 5 (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;
- 10 (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;
- 15 (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;
- 20 (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 SEQ ID NO:292;
- 25 (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;
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- (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;
- 5 (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;
- 10 (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 SEQ ID NO:292;
- 15 (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;
- 20 (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;
- 25 (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;
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- (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;
- (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.
11. The isolated monoclonal antibody or antigen-binding fragment thereof of any one of embodiments 1-10, wherein the monoclonal antibody or antigen-binding fragment thereof is capable of activating T cells.
12. An isolated nucleic acid encoding the monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-11.
13. A vector comprising the isolated nucleic acid of claim 12.
14. A host cell comprising the vector of claim 13.
15. A pharmaceutical composition, comprising the isolated monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-11 and a pharmaceutically acceptable carrier.
16. A method of inhibiting the nucleotidase activity of CD73 in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
17. 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.
18. 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.
19. A method of treating cancer in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
20. A method of producing the monoclonal antibody or antigen-binding fragment thereof of any one of claims 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.

21. A method of producing a pharmaceutical composition comprising the monoclonal antibody or antigen-binding fragment of any one of claims 1-11, comprising combining
5 the monoclonal antibody or antigen-binding fragment with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.
22. 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 an isolated monoclonal antibody or antigen-
10 binding fragment thereof of any one of claims 1-11; and
c. determining a level of CD73 in the subject.
23. The method of claim 22, wherein the sample is a tissue sample
24. The method of claim 23, wherein the tissue sample is a cancer tissue sample.
25. The method of claim 22, wherein the sample is a blood sample.
- 15 26. 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 claims 1-11; and
20 c. determining the ecto-5'-nucleotidase activity of CD73 in the subject.
27. The method of claim 26, wherein the sample is a tissue sample.
28. The method of claim 27, wherein the tissue sample is a cancer tissue sample.
29. The method of claim 26, wherein the sample is a blood sample.