

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:
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- (1) SEQ ID NOs:168, 169, 170, 171, 102, and 172, respectively;
- (2) SEQ ID NOs:50, 51, 52, 110, 111, and 112, respectively;
- (3) SEQ ID NOs:173, 174, 175, 176, 114, and 115, respectively;
- 10 (4) SEQ ID NOs:68, 177, 178, 128, 129, and 130, respectively;
- (5) SEQ ID NOs:74, 75, 76, 134, 135, and 136, respectively;
- (6) SEQ ID NOs:179, 78, 180, 137, 138, and 139, respectively;
- (7) SEQ ID NOs:83, 84, 85, 143, 144, and 145, respectively;
- (8) SEQ ID NOs:86, 87, 88, 146, 147, and 148, respectively;
- 15 (9) SEQ ID NOs:89, 90, 91, 149, 150, and 151, respectively;
- (10) SEQ ID NOs:92, 93, 94, 152, 153, and 154, respectively;
- (11) SEQ ID NOs:95, 96, 97, 155, 156, and 157, respectively; or
- (12) SEQ ID NOs:98, 99, 100, 158, 159, and 160, respectively;
- wherein the antibody or antigen-binding fragment thereof specifically binds apelin,
- 20 preferably human apelin.
2. The isolated monoclonal antibody or antigen-binding fragment of claim 1, comprising a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, or 39, or a light chain variable region having a polypeptide sequence at least 95%
- 25 identical to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, or 40.
3. The isolated monoclonal antibody or antigen-binding fragment of claim 1 or 2, comprising:
- a. a heavy chain variable region having the polypeptide sequence of SEQ ID
- 30 NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;

- b. 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;
- c. 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;
- d. 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;
- e. 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;
- f. 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;
- g. 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;
- h. 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;
- i. 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;
- j. 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;
- k. 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;

1. 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 m. 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;
- n. 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 o. 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;
- p. 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 q. 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;
- 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;
- 20 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
- 25 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.
4. An isolated monoclonal antibody or antigen-binding fragment thereof to apelin that specifically binds to an epitope comprising the amino acid sequence of SEQ ID NO:188.
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5. An isolated monoclonal antibody or antigen-binding fragment thereof to apelin that specifically binds to an epitope comprising the amino acid sequence of SEQ ID NO:204.
6. The isolated monoclonal antibody or antigen-binding fragment thereof of claim 4 or 5, wherein the antibody or antigen-binding fragment thereof inhibits apelin activity.
7. The isolated monoclonal antibody or antigen-binding fragment thereof of claim 4, wherein the antibody or antigen-binding fragment thereof is capable of binding apelin-13, pyro-apelin-13, apelin-17, apelin-36, apelin-55, and/or other forms of apelin that share the same C-terminal end with apelin-13.
8. The isolated monoclonal antibody or antigen-binding fragment thereof of claim 5, wherein the antibody or antigen-binding fragment thereof is capable of binding apelin-13 and pyro-apelin-13.
9. The isolated monoclonal antibody or antigen-binding fragment of any one of claims 1 to 8, wherein the antibody or antigen-binding fragment thereof is chimeric.
10. The isolated monoclonal antibody or antigen-binding fragment of any one of claims 1 to 9, wherein the antibody or antigen-binding fragment thereof is human or humanized.
11. The isolated monoclonal antibody or antigen-binding fragment of claim 10, wherein the antibody or antigen-binding fragment thereof comprises:
- a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:211, and a light chain variable region having the polypeptide sequence of SEQ ID NO:215;
 - b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:212, and a light chain variable region having the polypeptide sequence of SEQ ID NO:215;
 - c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:213, and a light chain variable region having the polypeptide sequence of SEQ ID NO:215;
 - d. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:214, and a light chain variable region having the polypeptide sequence of SEQ ID NO:215;

- e. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:213, and a light chain variable region having the polypeptide sequence of SEQ ID NO:216;
 - f. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:214, and a light chain variable region having the polypeptide sequence of SEQ ID NO:216;
 - g. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:217, and a light chain variable region having the polypeptide sequence of SEQ ID NO:219; or
 - h. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:218, and a light chain variable region having the polypeptide sequence of SEQ ID NO:220.
12. An isolated nucleic acid encoding the monoclonal antibody or antigen-binding fragment of any one of claims 1 to 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 of any one of claims 1 to 11 and a pharmaceutically acceptable carrier.
16. A method of blocking binding of apelin to an apelin receptor in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
17. A method of treating a diabetic retinopathy (DR) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
18. A method of treating an age-related macular degeneration (AMD) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
19. A method of treating a diabetic macular edema (DME) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.

20. A method of treating a macular edema following retinal vein occlusion (RVO) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
21. A method of treating a retinal degeneration in a subject in need thereof,
5 comprising administering to the subject the pharmaceutical composition of claim 15.
22. A method of treating a myopic choroidal neovascularization (mCNV) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
23. A method of treating a diabetic nephropathy in a subject in need thereof,
10 comprising administering to the subject the pharmaceutical composition of claim 15.
24. A method of treating a chronic kidney disease (CKD) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
25. A method of treating a non-alcoholic steatohepatitis (NASH) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim
15 15.
26. A method of treating a liver cirrhosis in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
27. A method of treating a plaque neovascularization in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
- 20 28. A method of treating a rubeosis iridis in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
29. A method of treating a neovascular glaucoma in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
30. A method of treating a corneal neovascularization (CNV) in a subject in need
25 thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
31. A method of treating a retinopathy of prematurity (ROP) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
- 30 32. A method of treating a retinopathy in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.

33. A method of treating a macular degeneration in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
34. A method of treating ovarian hyperstimulation syndrome (OHSS) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
35. A method of treating a uterine bleeding in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
36. A method of treating an endometriosis in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
37. A method of treating an endometrial hyperplasia and cancer in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
38. A method of treating a myometrial leiomyomas in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
39. A method of treating an adenomyosis in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
40. A method of treating a tissue fibrosis in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
41. A method of treating a cancer in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.
42. The method of claim 41, wherein the pharmaceutical composition further comprises a second anti-cancer agent.
43. The method of claim 42, wherein the second anti-cancer agent is an anti-VEGF agent.
44. The method of claim 43, wherein the anti-VEGF agent is Avastin or a bevacizumab biosimilar agent.
45. The method of claim 43, wherein the anti-VEGF agent is a VEGFR1 and/or VEGFR2 blocker.
46. A method of determining a level of apelin in a subject, the method comprising:
- obtaining a sample from the subject;

- b. contacting the sample with an antibody or antigen-binding fragment of any one of claims 1 to 11; and
 - c. determining a level of apelin in the subject.
47. The method of claim 46, wherein the sample is a tissue sample
- 5 48. The method of claim 47, wherein the tissue sample is a cancer tissue sample.
49. The method of claim 46, wherein the sample is a blood sample.
50. A method of producing the monoclonal antibody or antigen-binding fragment of any one of claims 1 to 11, comprising culturing a cell comprising a nucleic acid encoding the monoclonal antibody or antigen-binding fragment under conditions to produce the
- 10 monoclonal antibody or antigen-binding fragment, and recovering the antibody or antigen-binding fragment from the cell or culture.
51. A method of producing a pharmaceutical composition comprising the monoclonal antibody or antigen-binding fragment of any one of claims 1 to 11, comprising combining the monoclonal antibody or antigen-binding fragment with a pharmaceutically acceptable
- 15 carrier to obtain the pharmaceutical composition.