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:

(1) SEQ ID NOs:~~168, 169, 170, 171~~ 41, 42, 43, 101, 102, and ~~172~~103, respectively;

(2) SEQ ID NOs:~~50, 51, 52, 110, 111~~ 44, 45, 46, 104, 105, and ~~112~~106, respectively; or

(3) SEQ ID NOs:~~173, 174, 175, 176, 114~~ 47, 48, 49, 107, 08, and ~~115~~, respectively;

~~(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;~~

~~(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~~, preferably human apelin.~~.

2. The isolated monoclonal antibody or antigen-binding fragment of claim 1, comprising:

(a) 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~~and a light chain variable region having a polypeptide sequence at least 95% 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.~~;

~~The~~(b) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:3, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:4;

(c) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:5, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:6;

(d) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:213, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:216; or

(e) a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:214, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:216.

~~2.~~3. An isolated monoclonal antibody or antigen-binding fragment ~~of claim 1 or 2,~~thereof comprising:

a. 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;

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;~~

l. 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;

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;

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;

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;

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

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.

3. 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.

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:204.

5. 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.

6. 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.

~~7. 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.~~

~~8. 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.~~

~~9. 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.~~

~~10. The isolated monoclonal antibody or antigen-binding fragment of claim 10, wherein the antibody or antigen-binding fragment thereof comprises:~~

~~u.~~d. 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;

~~v.~~e. 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;

~~w.~~f. 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;

~~x.~~g. 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;

~~y.~~h. 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;

~~z.~~i. 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;

~~aa.~~j. 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

~~bb.~~k. 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.

4. The isolated monoclonal antibody or antigen-binding fragment thereof of claim 1 or 3, wherein the isolated monoclonal antibody or antigen-binding fragment thereof specifically binds to an epitope with the amino acid sequence of SEQ ID NO:188, wherein binding of the monoclonal antibody or antigen-binding fragment thereof to apelin inhibits apelin-mediated signaling through the apelin receptor and neutralizes apelin activity.

5. The isolated monoclonal antibody or antigen-binding fragment thereof of claim 4, wherein any one of the side chains of residues 11, 12, or 13 of apelin-13 is required for binding to the epitope comprising the amino acid sequence of SEQ ID NO:188.

6. The isolated monoclonal antibody or antigen-binding fragment thereof of claim 4, wherein the monoclonal antibody or antigen-binding fragment thereof binds to the C-terminal end of 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.

7. The isolated monoclonal antibody or antigen-binding fragment of any one of claims 1-6, wherein the antibody or antigen-binding fragment thereof is chimeric and/or humanized.

~~11.~~8. An isolated nucleic acid encoding the monoclonal antibody or antigen-binding fragment of any one of claims 1~~ to 11~~-7.

~~12.~~9. A vector comprising the isolated nucleic acid of claim ~~12~~8.

~~13.~~10. A host cell comprising the vector of claim ~~13~~9.

~~14.~~11. A pharmaceutical composition, comprising the isolated monoclonal antibody or antigen-binding fragment of any one of claims 1~~ to 11~~-7 and a pharmaceutically acceptable carrier.

~~15.~~12. ~~A~~The pharmaceutical composition of claim 11 for use in a method of blocking binding of apelin to an apelin receptor in a subject in need thereof, ~~comprising administering~~wherein the pharmaceutical composition is to be administered to the subject~~ the pharmaceutical composition of claim 15~~.

~~16. A method of treating a diabetic retinopathy (DR) in a subject in need thereof, comprising administering to the subject~~Use of the pharmaceutical composition of claim ~~15.~~

~~17. A method of treating~~11 in the manufacture of a medicament for the treatment of a disease selected from the group consisting of a diabetic retinopathy (DR), an age-related macular

degeneration (AMD~~) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~18. 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.~~

~~19. 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.~~

~~20. A method of treating~~), a retinal degeneration ~~in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~21. 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.~~

~~22. A method of treating~~), a diabetic nephropathy ~~in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~23. 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.~~

~~24. 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.~~

~~25. A method of treating~~), a liver cirrhosis ~~in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~26. A method of treating~~, a plaque neovascularization ~~in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~27. A method of treating~~, a rubeosis iridis ~~in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~28. A method of treating~~, a neovascular glaucoma ~~in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~29. A method of treating~~, a corneal neovascularization (CNV) ~~in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~30. 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.~~

~~31. A method of treating~~), a retinopathy ~~in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~32. A method of treating~~, a macular degeneration ~~in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~33. A method of treating~~ , an ovarian hyperstimulation syndrome (OHSS~~) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~34. A method of treating~~), a uterine bleeding ~~in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~35. A method of treating~~, an endometriosis ~~in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~36.~~13. ~~A method of treating~~, an endometrial hyperplasia, a myometrial leiomyomas, an adenomyosis, a tissue fibrosis, and a cancer in a subject in need thereof, ~~comprising administering to the subject~~wherein the pharmaceutical composition ~~of claim 15~~is to be administered to the subject.

~~37. A method of treating a myometrial leiomyomas in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~38. A method of treating an adenomyosis in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~39. A method of treating a tissue fibrosis in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~40. A method of treating a~~ The use of claim 13, wherein the disease is cancer ~~in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 15.~~

~~41.~~14. ~~The method of claim 41, wherein~~and the pharmaceutical composition further comprises ~~a second~~an anti-cancer agent.

~~42.~~ The ~~method~~use of claim ~~42~~14, wherein the ~~second~~ anti-cancer agent is an anti-VEGF agent~~.~~

~~43. The method of claim 43~~, wherein the anti-VEGF agent is ~~Avastin or a~~selected from an anti-VEGF antibody, bevacizumab ~~biosimilar agent.~~

~~44.~~15. ~~The method of claim 43, wherein the anti-VEGF agent is a VEGFR1 and/or VEGFR2 blocker~~, randibizumab, aflibercept, or conbercept.

~~45.~~16. A method of determining a level of apelin in a subject, the method comprising:

a. obtaining a sample from the subject;

b. contacting the sample with ~~an~~a monoclonal antibody or antigen-binding fragment of any one of claims 1~~ to 11~~ 7; and

c. determining a level of apelin in the subject.

~~46.~~17. The method of claim ~~46~~16, wherein the sample is a tissue sample or a blood sample.

~~47.~~18. The method of claim ~~47~~17, wherein the tissue sample is a cancer tissue sample.

~~48. The method of claim 46, wherein the sample is a blood sample.~~

~~49.~~19. A method of producing the monoclonal antibody or antigen-binding fragment of any one of claims 1~~ to 11~~ 7, comprising culturing a cell comprising a nucleic acid encoding the monoclonal antibody or antigen-binding fragment of any one of claims 1-7 under conditions to produce the monoclonal antibody or antigen-binding fragment, and recovering the antibody or antigen-binding fragment from the cell or culture.

20. ~~A method of producing a pharmaceutical composition comprising the~~The isolated monoclonal antibody or antigen-binding fragment thereof of ~~any one of claims~~claim 1 ~~to 11, comprising combining~~or 3, wherein the monoclonal antibody or antigen-binding fragment ~~with~~ thereof specifically binds human apelin.

~~50.~~21. The pharmaceutical composition of claim 11 for use in treating a disease selected from the group consisting of a ~~pharmaceutically acceptable carrier to obtain~~diabetic retinopathy (DR), an age-related macular degeneration (AMD), a diabetic macular edema (DME), a macular edema following retinal vein occlusion (RVO), a retinal degeneration, a myopic choroidal neovascularization (mCNV), a diabetic nephropathy, a chronic kidney disease (CKD), a non-alcoholic steatohepatitis (NASH), a liver cirrhosis, a plaque neovascularization, a rubeosis iridis, a neovascular glaucoma, a corneal neovascularization (CNV) a retinopathy of prematurity (ROP), a retinopathy, a macular degeneration, an ovarian hyperstimulation syndrome (OHSS), a uterine bleeding, an endometriosis, an endometrial hyperplasia, a myometrial leiomyomas, an adenomyosis, a tissue fibrosis, and a cancer in a subject in need thereof, wherein the pharmaceutical composition is to be administered to the subject.

22. The pharmaceutical composition for use of claim 21, wherein the disease is cancer and the pharmaceutical composition further comprises an anti-cancer agent.

~~51.~~23. The pharmaceutical composition for use of claim 21, wherein the anti-cancer agent is an anti-VEGF agent, wherein the anti-VEGF agent is selected from an anti-VEGF antibody, bevacizumab, randibizumab, aflibercept, or conbercept.