

Claims

We claim:

1. An isolated antibody or antigen-binding fragment thereof that binds human cannabinoid type 1 receptor (CB1) (SEQ ID NO:1), wherein the binding protein comprises six complementarity determining regions (CDRs): CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, wherein

CDR-H1 has an amino acid sequence G-Y-T-F-T-D-Y-W (residues 26-33 of SEQ ID NO:329) or a modification of said amino acid sequence by a substitution of at least one amino acid residue, wherein the substitution of G at position 1 is S; the substitution of T at position 3 is E; the substitution of T at position 5 is S or N; the substitution of D at position 6 is R or Y; the substitution of Y at position 7 is H; and the substitution of W at position 8 is A or N;

CDR-H2 has an amino acid sequence I-Y-P-Y-D-G-D-T (residues 51-58 of SEQ ID NO:329) or a modification of said amino acid sequence by a substitution of at least one amino acid residue, wherein the substitution of I at position 1 is F; the substitution of Y at position 2 is D, S, or T; the substitution of P at position 3 is T; the substitution of Y at position 4 is G, D, or S; the substitution of D at position 5 is Y or S; the substitution of G at position 6 is S; the substitution of D at position 7 is E, G, or R; and the substitution of T at position 8 is A, S, or I;

CDR-H3 has an amino acid sequence A-R-G-X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-W-X₁₀-X₁₁-Y (residues 98-113 of SEQ ID NO:329) or a modification of said amino acid sequence by substitution of at least one amino acid residue, wherein the substitution of A at position 1 is S; the substitution of G at position 3 is S; X₁ at position 4 is Q, Y, K, R, or G, or is not present; X₂ at position 5 is E, Y, L or G, or is not present; X₃ at position 6 is Y or P, or is not present; X₄ at position 7 is Y, R, or E, or is not present; X₅ at position 8 is G, or is not present; X₆ at position 9 is T, or is not present; X₇ at position 10 is N or D, or is not present; X₈ at position 11 is Y, N, A, or G, or is not present; X₉ at position 12 is N, Y, S, A, or R, or is not present; the substitution of W at position 13 is Y, A, or P; X₁₀ at position 14 is L, M, F, or G, or is not present; X₁₁ at position 15 is P, D, A, or T, or is not present; the substitution of Y at position 16 is V;

CDR-L1 has an amino acid sequence Q-X₁-I-S-S-X₂-Y (residues 27-33 of SEQ ID NO:330) or a modification of said amino acid sequence by substitution of at least one amino acid residue, wherein the substitution of Q at position 1 is S or E; X₁ at position 2 is E, S, T,

N, G, or R; the substitution of I at position 3 is V; the substitution of S at position 4 is A, R, or G; the substitution of S at position 5 is G, N, or T; X₂ at position 6 is S, N, the peptide F-R-Y-S, or is not present; and the substitution of Y at position 7 is F, D, or N;

CDR-L2 has an amino acid sequence: X₁-T-S (residues 51-53 of SEQ ID NO:330) or
 5 a modification of said amino acid sequence by substitution of at least one amino acid residue, wherein X₁ at position 1 is A, Y, G, R, D, or S; the substitution of T at position 2 is A; the substitution of S at position 3 is R; and

CDR-L3 has an amino acid sequence: Q-Q-Y-X₁-S-X₂-P-Y-T (residues 91-99 of SEQ ID NO:330) or a modification of said amino acid sequence by substitution of at least one
 10 amino acid residue, wherein the substitution of Q at position 1 is L or H; the substitution of Q at position 2 is H; the substitution of Y at position 3 is S or G; X₁ at position 4 is S, W, H, Y, N, or I; the substitution of S at position 5 is E, R, G, T, or N; X₂ at position 6 is Y, I, S, T, L, or W; and the substitution of Y at position 8 is P, L, F, or is not present; and wherein said substitution, addition, or deletion of at least one amino acid residue does not inhibit the
 15 ability of said antibody or antigen-binding fragment thereof to bind human CB1.

2. An isolated antibody or antigen-binding fragment thereof that binds human cannabinoid type 1 receptor (CB1) (SEQ ID NO:1) wherein the antibody comprises CDRs of a variable heavy (VH) domain sequence and CDRs of a variable light (VL) domain sequence, wherein
 20 the VH domain sequence is selected from the group consisting of SEQ ID NOs: 18, 30, 42, 54, 66, 78, 90, 102, 114, 126, 138, 150, 162, 174, 186, 198, 210, 222, 234, 246, 258, 270, 282, 294, 306, and 318, and/or wherein the VL domain is selected from the group consisting of SEQ ID NOs: 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180, 192, 204, 216, 228, 240, 252, 264, 276, 288, 300, 312, and 324.

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 3. An isolated antibody or antigen-binding fragment thereof that binds human cannabinoid type 1 receptor (CB1) (SEQ ID NO:1) wherein the antibody comprises a variable heavy (VH) domain sequence and a variable light (VL) domain sequence, wherein the VH domain sequence is selected from the group consisting of SEQ ID NOs: 18, 30, 42, 54, 66, 78, 90,
 30 102, 114, 126, 138, 150, 162, 174, 186, 198, 210, 222, 234, 246, 258, 270, 282, 294, 306, and 318, and/or wherein the VL domain is selected from the group consisting of SEQ ID NOs: 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180, 192, 204, 216, 228, 240, 252, 264, 276, 288, 300, 312, and 324.

4. The isolated antibody or antigen-binding fragment thereof of claim 3, wherein the antibody or antigen-binding fragment thereof comprises the heavy and light chain CDRs of a VH/VL pair selected from the group consisting of SEQ ID NOs: 18/24, 30/36, 42/48, 54/60, 66/72, 78/84, 90/96, 102/108, 114/120, 126/132, 138/144, 150/156, 162/168, 174/180, 186/192, 198/204, 210/216, 222/228, 234/240, 246/252, 258/264, 270/276, 282/288, 294/300, 306/312, and 318/324.

5. The isolated antibody or antigen-binding fragment thereof of claim 4, wherein the antibody or antigen-binding fragment thereof comprises the VH/VL pair selected from the group consisting of: SEQ ID NOs: 18/24, 30/36, 42/48, 54/60, 66/72, 78/84, 90/96, 102/108, 114/120, 126/132, 138/144, 150/156, 162/168, 174/180, 186/192, 198/204, 210/216, 222/228, 234/240, 246/252, 258/264, 270/276, 282/288, 294/300, 306/312, and 318/324.

6. The isolated antibody or antigen-binding fragment thereof of claim 3, wherein the antibody or antigen-binding fragment thereof comprises an HCDR set (HCDR1, HCDR2, HCDR3) selected from the group consisting of (20, 21, 22); (32, 33, 34); (44, 45, 46); (56, 57, 58); (68, 69, 70); (80, 81, 82); (92, 93, 94); (104, 105, 106); (116, 117, 118); (128, 129, 130); (140, 141, 142); (152, 153, 154); (164, 165, 166); (176, 177, 178); (188, 189, 190); (200, 201, 202); (212, 213, 214); (224, 225, 226); (236, 237, 238); (248, 249, 250); (260, 261, 262); (272, 273, 274); (284, 285, 286); (296, 297, 298); (308, 309, 310); and (320, 321, 322) and an LCDR set (LCDR1, LCDR2, LCDR3) selected from the group consisting of (26, 27, 28); (38, 39, 40); (50, 51, 52); (62, 63, 64); (74, 75, 76); (86, 87, 88); (98, 99, 100); (110, 111, 112); (122, 123, 124); (134, 135, 136); (146, 147, 148); (158, 159, 160); (170, 171, 172); (182, 183, 184); (194, 195, 196); (206, 207, 208); (218, 219, 220); (230, 231, 232); (242, 243, 244); (254, 255, 256); (266, 267, 268); (278, 279, 280); (290, 291, 292); (302, 303, 304); (314, 315, 316); and (326, 327, 328).

7. The isolated antibody or antigen-binding fragment thereof of claim 6, wherein the antibody or antigen-binding fragment thereof comprises the (HCDR set/LCDR set) pair selected from the group consisting of SEQ ID NOs: (20, 21, 22/ 26, 27, 28); (32, 33, 34/ 38, 39, 40); (44, 45, 46/ 50, 51, 52); (56, 57, 58/ 62, 63, 64); (68, 69, 70/ 74, 75, 76); (80, 81, 82/ 86, 87, 88); (92, 93, 94/ 98, 99, 100); (104, 105, 106/ 110, 111, 112); (116, 117, 118/ 122, 123, 124); (128, 129, 130/ 134, 135, 136); (140, 141, 142/ 146, 147, 148); (152, 153, 154/ 158, 159, 160); (164, 165, 166/ 170, 171, 172); (176, 177, 178/ 182, 183, 184); (188, 189,

190/ 194, 195, 196); (200, 201, 202/ 206, 207, 208); (212, 213, 214/ 218, 219, 220); (224, 225, 226/ 230, 231, 232); (236, 237, 238/ 242, 243, 244); (248, 249, 250/ 254, 255, 256); (260, 261, 262/ 266, 267, 268); (272, 273, 274/ 278, 279, 280); (284, 285, 286/ 290, 291, 292); (296, 297, 298/ 302, 303, 304); (308, 309, 310/ 314, 315, 316); and (320, 321, 322/ 326, 327, 328).

8. The isolated antibody or antigen-binding fragment thereof of claim 7, wherein the antibody or antigen-binding fragment thereof comprises the HC/LC pair selected from the group consisting of SEQ ID NOs: 17/23, 29/35, 41/47, 53/59, 65/71, 77/83, 89/95, 101/107, 113/119, 125/131, 137/143, 149/155, 161/167, 173/179, 185/191, 197/203, 209/215, 221/227, 233/239, 245/251, 257/263, 269/275, 281/287, 283/289, 305/311, and 317/323.

9. An isolated antibody or antigen-binding fragment thereof that binds human cannabinoid type 1 receptor (CB1) (SEQ ID NO:1) wherein the antibody comprises CDRs of a variable heavy (VH) domain sequence and CDRs of a variable light (VL) domain sequence, wherein the VH domain sequence has at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 30, 42, 54, 66, 78, 90, 102, 114, 126, 138, 150, 162, 174, 186, 198, 210, 222, 234, 246, 258, 270, 282, 294, 306, and 318 and/or the VL domain sequence having at least 95% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180, 192, 204, 216, 228, 240, 252, 264, 276, 288, 300, 312, and 324.

10. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 114 and the VL is set forth in SEQ ID NO: 120.

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11. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 126 and the VL is set forth in SEQ ID NO: 132.

12. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 138 and the VL is set forth in SEQ ID NO: 144.

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13. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 150 and the VL is set forth in SEQ ID NO: 156.

14. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 162 and the VL is set forth in SEQ ID NO: 168.
15. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 174 and the VL is set forth in SEQ ID NO: 180.
16. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 186 and the VL is set forth in SEQ ID NO: 192.
17. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 198 and the VL is set forth in SEQ ID NO: 204.
18. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 210 and the VL is set forth in SEQ ID NO: 216.
19. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 222 and the VL is set forth in SEQ ID NO: 228.
20. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 234 and the VL is set forth in SEQ ID NO: 240.
21. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 246 and the VL is set forth in SEQ ID NO: 252.
22. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 258 and the VL is set forth in SEQ ID NO: 264.
23. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 270 and the VL is set forth in SEQ ID NO: 276.
24. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 282 and the VL is set forth in SEQ ID NO: 288.
25. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is

set forth in SEQ ID NO: 294 and the VL is set forth in SEQ ID NO: 300.

26. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the VH is set forth in SEQ ID NO: 306 and the VL is set forth in SEQ ID NO: 312.

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27. The isolated antibody or antigen-binding fragment thereof of claim 1, wherein the VH is set forth in SEQ ID NO: 318 and the VL is set forth in SEQ ID NO: 324.

28. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-27,
10 wherein the antibody is a human or humanized antibody.

29. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-28,
wherein the fragment comprises a Fab fragment, a Fab' fragment, a F(ab)₂ fragment or a scFv
fragment.

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30. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-29,
wherein the antibody or antigen-binding fragment thereof comprises a human Fc region
selected from the group consisting of an IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, and IgM Fc.

20 31. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-29,
wherein the antibody or antigen-binding fragment thereof comprises a modified human Fc
region.

32. The isolated antibody or antigen-binding fragment thereof of claim 31, wherein the
25 antibody or antigen-binding fragment thereof comprises a modified human Fc region
comprising a mutation selected from the group consisting of L234A/L235A, S228P, A330S,
P331S, E233P/L234V/L235A, A327G/A330S/P331S, L234F/L235E/P331S, and N297Q.

33. A multispecific binding protein comprising an antigen-binding fragment of any one of
30 claims 1-32.

34. The isolated antibody or antigen-binding fragment of any one of claims 1-33, wherein
the antibody or antigen-binding fragment thereof inhibits CB1 signaling activity.

35. The isolated antibody or antigen-binding fragment of any one of claims 1-33, wherein the antibody or antigen-binding fragment thereof enhances or activates CB1 signaling activity.
- 5 36. The isolated antibody or antigen-binding fragment of any one of claims 1-33, wherein the antibody or antigen-binding fragment thereof is an inverse agonist to CB1 signaling activity.
37. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-36,
10 wherein the antibody or antigen-binding fragment thereof is a humanized antibody.
38. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-36, wherein the antibody or antigen-binding fragment thereof is a fully human antibody.
- 15 39. An isolated antibody or antigen-binding fragment thereof that competes for binding to CB1 with an isolated anti-human CB1 antibody or antigen-binding fragment thereof of any one of claims 1-38.
40. The isolated antibody or antigen-binding fragment thereof of claim 39, wherein the
20 antibody or antigen-binding fragment thereof that competes for binding is a human or humanized antibody.
41. An antibody or antigen-binding fragment thereof that specifically binds to substantially the same epitope of CB1 as the antibody or antigen-binding fragment of any one of claims 1-
25 38.
42. The isolated antibody or antigen-binding fragment thereof of claim 41, wherein the antibody or antigen-binding fragment thereof that specifically binds to substantially the same epitope of CB1 is a human or humanized antibody.
- 30 43. An isolated antibody or antigen-binding fragment thereof that binds to cannabinoid type 1 receptor (CB1), wherein the antibody or antigen-binding fragment thereof has a binding affinity K_d for CB1 of about 1 μ M or less.

44. The isolated antibody or antigen-binding fragment of claim 43, wherein the antibody or antigen-binding fragment thereof has a binding affinity K_d for CB1 of about 100 nM or less.
45. The isolated antibody or antigen-binding fragment of any one of claims 1-44, wherein
5 the antibody or antigen-binding fragment exhibits reduced brain penetration as compared to rimonabant.
46. The isolated antibody or antigen-binding fragment of any one of claims 1-44, wherein
10 the antibody or antigen-binding fragment inhibits CB1 signaling that is at least 2 fold higher as compared to rimonabant.
47. The isolated antibody or antigen-binding fragment of any one of claims 1-44, wherein
15 the antibody or antigen-binding fragment exhibits reduced CNS side effects relative to rimonabant.
48. An isolated nucleic acid molecule encoding an antibody or antigen-binding fragment
thereof of any one of claims 1-47.
49. An expression vector comprising the nucleic acid molecule of claim 48.
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50. A host cell comprising the expression vector of claim 49.
51. A method of modulating CB1 signaling, the method comprising contacting a cell
expressing CB1 with the antibody or antigen-binding fragment thereof of any one of claims
25 1-47.
52. A method of antagonizing CB1, the method comprising contacting a cell expressing CB1
with the antibody or antigen-binding fragment thereof of any one of claims 1-47.
- 30 53. A method of agonizing CB1, the method comprising contacting a cell expressing CB1
with the antibody or antigen-binding fragment thereof of any one of claims 1-47.
54. A method of inverse agonizing CB1, the method comprising contacting a cell expressing
CB1 with the antibody or antigen-binding fragment thereof of any one claims 1-47.

55. A pharmaceutical composition comprising an isolated antibody or antigen-binding fragment thereof of any one of claims 1-47 and at least one pharmaceutically acceptable excipient.
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56. A method for inhibiting the biological activity of CB1 in a subject in need thereof, the method comprising administering an effective amount of the pharmaceutical composition of claim 55 to the subject thereby inhibiting the activity of the CB1 protein in the subject.
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57. A method for treating a disease associated with CB1 activity, the method comprising administering the pharmaceutical composition of claim 55 to a subject afflicted with the disease.
58. A method of treating a disease or disorder responsive to modulation of CB1 signaling in a subject in need thereof, the method comprising administering to the pharmaceutical composition of claim 55.
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59. A method of treating a disease or disorder responsive to antagonism or inverse agonism of CB1 signaling in a subject in need thereof, the method comprising administering to the subject the pharmaceutical composition of claim 55.
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60. A method of treating a disease or disorder responsive to agonism of CB1 signaling in a subject in need thereof, the method comprising administering to the subject the pharmaceutical composition of claim 55.
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61. A method for diagnosing a disease or disorder associated with CB1, the method comprising contacting a cell with an antibody or antigen-binding fragment thereof of any one of claims 1-47.
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62. The method of claim 61, wherein the disease or disorder is selected from the group consisting of obesity, syndromic obesities including Prader-Willi syndrome (PWS), Alström syndrome, Bardet-Biedel syndrome (BBS), Albright Hereditary Osteodystrophy (AHO), and SIM1 deletion syndrome; diabetes and related complications; dyslipidemia; liver diseases such as, for example, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease,

and primary biliary cirrhosis; fibrosis, for example, kidney fibrosis; chronic kidney disease; diabetic neuropathy, focal segmental glomerulosclerosis, renal disease; metabolic diseases, osteoporosis, atherosclerosis, inflammatory disease, cardiovascular disease, cancer, pain, systemic sclerosis, multiple sclerosis spasticity, glaucoma, and nicotine addiction.

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63. An antibody conjugate comprising the isolated antibody or antigen-binding fragment thereof of any one of claims 1-47, wherein the antibody or antigen-binding fragment thereof is conjugated to an agent selected from the group consisting of a therapeutic agent, a cytotoxic agent, an immunoadhesion molecule, and an imaging agent.

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