ANTI-ROBO4-ANTIBODY

The present invention relates to an antibody having an anti-angiogenesis activity. More specifically, the present invention relates to an antibody against ROBO4 and a pharmaceutical composition containing the antibody.

[Background Art]

Roundabout homolog 4 (ROBO4), a protein of 110 kDa in molecular weight, has a single-pass transmembrane structure (Non Patent Literature 1) and is known to suppress angiogenesis through the binding to a known angiogenesis suppressor Slit homolog 2 (Slit2) (Patent Literature 1 and Non Patent Literature 2). Slit2 has been reported to suppress the migration of HUVEC promoted by a vascular endothelial growth factor (VEGF) and has also been reported to suppress the promotion of cell migration by VEGF or bFGF for ROBO4 gene-transfected vascular endothelial cells (hereinafter, the vascular endothelial cell is also referred to as "EC") compared with empty vector-transfected EC (Patent Literature 1 and Non Patent Literatures 2 to 4).

Moreover, it has been reported that the suppressive effect of Slit2 on the promotion of cell migration, promotion of lumen formation, or increase in permeability by VEGF is observed in EC derived from a wild-type mouse or EC transfected with control siRNA, but not observed in EC derived from a ROBO4 gene-knockout mouse or EC transfected with siRNA to knock-down the ROBO4 gene (Patent Literature 2 and Non Patent Literatures 4 to 6). Furthermore, it has been reported that Slit2 suppresses, via ROBO4, angiogenesis or increase in vascular permeability in mouse models with laser-induced choroidal neovascularization or oxygen-induced retinopathy, which are animal disease models with exudative age-related macular degeneration or diabetic retinopathy (Patent Literature 2 and Non Patent Literature 4).

In spite of these findings, there are also reports showing that ROBO4 does not bind to Slit2 (Non Patent Literature 9 and Patent Literature 5). It has also been reported as to its functions that ROBO4 participates in the promotion of angiogenesis rather than the suppression of angiogenesis, because the migration or lumen formation of ROBO4 gene-knockout EC is inhibited (Non Patent Literatures 10 and 11).

In clinical practice, ROBO4 has been reported to be highly expressed in intratumoral vessels in liver metastasis from colon cancer, ganglioglioma, bladder cancer, breast cancer, metastatic melanoma, kidney cancer, lung cancer, liver cancer, or colon cancer (Patent Literature 3 and Non Patent Literatures 1, 3, and 7). Moreover, ROBO4 has been reported to be also expressed in blood vessels in the fibrovascular membranes of proliferative diabetic retinopathy patients (Non Patent Literature 8). As such, ROBO4 is expressed in vascular endothelial cells, particularly, endothelial cells in blood vessels newly formed in a pathological condition. This may suggest a pathological angiogenesis resulting from the high expression of ROBO4, but may also suggest the compensatory expression of ROBO4 for suppressing pathological angiogenesis.

As described above, ROBO4 is involved in an anti-angiogenesis effect. Thus, an antibody against ROBO4 and a functional fragment thereof are presumably useful in the treatment of a disease involving angiogenesis. However, it is uncertain whether either an agonistic or antagonistic antibody against ROBO4 suppresses or promotes angiogenesis.

Antibodies described in EP Patent No. 1,565,491 (Patent Literature 4) and WO2008/100805 (Patent Literature 5) are known as the antibody against ROBO4 (hereinafter, referred to as an "anti-ROBO4 antibody"). But none of these antibodies does not show a suppressive or inhibitory effect on angiogenesis *in vivo*.

[Citation List]

[Patent Literature]

[Patent Literature 1] WO2004/003163

[Patent Literature 2] WO2008/073441

[Patent Literature 3] WO2002/036771

[Patent Literature 4] European Patent No. 1,565,491

[Patent Literature 5] WO2008/100805

[Non Patent Literature]

[Non Patent Literature 1] Genomics, 2002, vol. 79, p. 547-552

[Non Patent Literature 2] Developmental Biology, 2003, vol. 261, p. 251-267

[Non Patent Literature 3] Biochemical and Biophysical Research Communications, 2005, vol. 332, p. 533-541

[Non Patent Literature 4] Nature Medicine, 2008, No. 14, p. 448-453

[Non Patent Literature 5] Science Translational Medicine, 2010, vol. 2, p. 23ra19

[Non Patent Literature 6] Proceedings of the National Academy of Sciences, 2010, vol. 107, p. 10520-10525

[Non Patent Literature 7] Oncology Reports, 2006, vol. 15, p.1437-1443

[Non Patent Literature 8] Molecular Vision, 2009, vol. 15, p. 1057-1069

[Non Patent Literature 9] The FASEB Journal, 2005, vol. 19, p. 121-123

[Non Patent Literature 10] BMC Cell Biology, 2008, vol. 9, p. 61-72

[Non Patent Literature 11] The FASEB Journal, 2009, vol. 23, p. 513-522

[Summary of Invention]

[Problems to be Solved by the Invention]

An object of the present invention is to provide an antibody against ROBO4.

A further object of the present invention is to provide a pharmaceutical composition containing an anti-ROBO4 antibody having an anti-angiogenesis effect, etc.

A further object of the present invention is to provide a method for producing the antibody.

A further object of the present invention is to provide a method for suppressing angiogenesis using the antibody, etc.

[Means for Solving the Problems]

The present inventors have conducted diligent studies to attain the objects and consequently successfully constructed a screening system that detects the activation of the downstream signal of ROBO4. Moreover, the present inventors have used the screening system to successfully obtain a novel anti-ROBO4 monoclonal antibody that activates the downstream signal of ROBO4, has a suppressive activity against cell migration induced by various angiogenic factors such as VEGF, bFGF, HGF, PDGF-BB and SDF-1 in ROBO4-expressing EC, and exhibits an anti-angiogenesis effect even in *in-vivo* models. In this way, the present invention has been completed.

Specifically, the present invention relates to:

(1) an antibody having properties described in the following (I) to (III) or a functional fragment thereof:

(I) binding to the ROBO4 protein, preferably with a K_D value of 1×10^{-8} or lower, specifically preferred 5×10^{-9} or lower;

(II) suppressing or inhibiting vascular endothelial cell migration in the absence of a cross-linking antibody *in vitro*; and

(III) suppressing or inhibiting angiogenesis *in vivo*;

(2) the antibody or the functional fragment thereof according to (1), wherein the ROBO4 protein is the human ROBO4 protein;

(3) the antibody or the functional fragment thereof according to (1), wherein the ROBO4 protein is a protein consisting of an amino acid sequence of amino acid Nos. 1 to 1007 of SEQ ID NO: 2 ;

(4) the antibody or the functional fragment thereof according to (1), wherein the ROBO4 protein is a protein consisting of an amino acid sequence of amino acid Nos. 46 to 1007 of SEQ ID NO: 2 ;

(5) the antibody or the functional fragment thereof according to (3) or (4), wherein the antibody or the functional fragment thereof binds to a site consisting of an amino acid sequence of amino acid Nos. 132 to 209 of SEQ ID NO: 2;

(6) the antibody or the functional fragment thereof according to (1), wherein the antibody or the functional fragment thereof is a monoclonal antibody or a functional fragment thereof;

(7) the antibody or the functional fragment thereof according to any one of (1) to (6), wherein the antibody consists of a heavy chain comprising CDRH1 consisting of the amino acid sequence represented by SEQ ID NO: 44 (Figure 25), CDRH2 consisting of the amino acid sequence represented by SEQ ID NO: 46 (Figure 26) or an amino acid sequence derived from the amino acid sequence of SEQ ID NO: 46 by the substitution of one amino acid, and CDRH3 consisting of the amino acid sequence represented by SEQ ID NO: 48 (Figure 27), and a light chain comprising CDRL1 consisting of the amino acid sequence represented by SEQ ID NO: 50 (Figure 28) or an amino acid sequence derived from the amino acid sequence of SEQ ID NO: 50 by the substitution of one to three amino acid(s), CDRL2 consisting of the amino acid sequence represented by SEQ ID NO: 52 (Figure 29), and CDRL3 consisting of the amino acid sequence represented by SEQ ID NO: 54 (Figure 30);

(8) the antibody or the functional fragment thereof according to any one of (1) to (7), wherein the antibody consists of a heavy chain comprising CDRH1 consisting of the amino acid sequence represented by SEQ ID NO: 44 (Figure 25), CDRH2 consisting of the amino acid sequence represented by SEQ ID NO: 46 (Figure 26) or the amino acid sequence represented by SEQ ID NO: 68 (Figure 44), and CDRH3 consisting of the amino acid sequence represented by SEQ ID NO: 48 (Figure 27), and a light chain comprising CDRL1 consisting of the amino acid sequence represented by SEQ ID NO: 50 (Figure 28) or the amino acid sequence represented by SEQ ID NO: 70 (Figure 46), CDRL2 consisting of the amino acid sequence represented by SEQ ID NO: 52 (Figure

29), and CDRL3 consisting of the amino acid sequence represented by SEQ ID NO: 54 (Figure 30);

(9) the antibody or the functional fragment thereof according to any one of (1) to (7), wherein the antibody comprises a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 31 (Figure 16), and a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 33 (Figure 18);

(10) the antibody or the functional fragment thereof according to any one of (1) to (8), wherein the antibody comprises any one heavy chain variable region selected from the following a) to d) and a light chain variable region selected from e) and f):

a) a heavy chain (hMAb1-H1-type) variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 56 (Figure 32),

b) a heavy chain (hMAb1-H2-type) variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 58 (Figure 34),

c) a heavy chain (hMAb1-H3-type) variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 60 (Figure 36) , and

d) a heavy chain (hMAb1-H4-type) variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 62 (Figure 38) ; and

e) a light chain (hMAb1-L1-type) variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40) , and

f) a light chain (hMAb1-L2-type) variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 66 (Figure 42) ;

(11) the antibody or the functional fragment thereof according to any one of (1) to (8), wherein the antibody comprises any one of the following combinations 1) to 6) of a heavy chain variable region and a light chain variable region:

1) a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 58 (Figure 34) and a light

chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40),

2) a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 58 (Figure 34) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 66 (Figure 42),

3) a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 62 (Figure 38) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 66 (Figure 42) ;

4) a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 62 (Figure 38) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40) ,

5) a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 56 (Figure 32) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40) , and

6) a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 60 (Figure 36) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40) ,

(12) the antibody or the functional fragment thereof according to any one of (1) to (11), wherein the antibody or the functional fragment thereof is a chimeric antibody or a functional fragment thereof;

(13) the antibody or the functional fragment thereof according to any one of (1) to (11), wherein the antibody or the functional fragment thereof is a humanized antibody or a functional fragment thereof;

(14) the antibody or the functional fragment thereof according to any one of (1) to (13), wherein the antibody comprises a human IgG1 or human IgG2 heavy chain constant region;

(15) the antibody or the functional fragment thereof according to any one of (1) to (6), wherein the antibody or the functional fragment thereof binds to a site on an antigen recognized by any one antibody according to (7) to (14);

(16) the antibody or the functional fragment thereof according to any one of (1) to (6), wherein the antibody or the functional fragment thereof competes with any one antibody according to (7) to (14) for the binding to ROBO4 protein;

(17) the antibody according to any one of (1) to (8), wherein the antibody comprises any one of the following combinations 1) to 6) of a heavy chain and a light chain:

1) a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 58 (Figure 34) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 64 (Figure 40) (H-1140),

2) a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 58 (Figure 34) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 66 (Figure 42) (H-1143),

3) a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 62 (Figure 38) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 66 (Figure 42) (H-2143);

4) a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 62 (Figure 38) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 64 (Figure 40) (H-2140),

5) a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 56 (Figure 32) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 64 (Figure 40) (H-1040), and

6) a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 60 (Figure 36) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 64 (Figure 40) (H-2040),

(18) an antibody comprising a modified form of the heavy chain of any one antibody according to (17) , wherein said modified form lacks one to several carboxyl-terminal amino acid(s) of said heavy chain, preferably one to eight carboxyl-terminal amino acid(s) of said heavy chain, more preferably one to two carboxyl-terminal amino acid(s) of said heavy chain;

(19) the antibody according to any one of (1) to (6), wherein the antibody is 95% or more identical in the amino acid sequence to any one antibody according to (17) ; has a K_D value of 1×10^{-8} or lower for a human ROBO4; suppresses or inhibits vascular endothelial cell migration in the absence of a cross-linking antibody *in vitro*; and suppresses or inhibits angiogenesis *in vivo*.;

(20) the antibody or the functional fragment thereof according to (15), wherein the antibody or the functional fragment thereof is a human antibody or a functional fragment thereof;

(21) a nucleotide sequence selected from the group consisting of the following (I) to (III):

(I) a nucleotide sequence comprising a nucleotide sequence encoding the partial or whole amino acid sequence of the heavy chain or light chain of an antibody according to any one of (1) to (20);

(II) a nucleotide sequence consisting of a nucleotide sequence comprising a nucleotide sequence encoding the partial or whole amino acid sequence of the heavy chain or light chain of an antibody according to any one of (1) to (20); and

(III) a nucleotide sequence consisting of a nucleotide sequence encoding the partial or whole amino acid sequence of the heavy chain or light chain of an antibody according to any one of (1) to (20);

(22) a recombinant vector containing an insert of a nucleotidesequence according to (21);

(23) a recombinant cell containing a nucleotide according to (21) or a recombinant vector according to (22) introduced therein;

(24) a cell producing an antibody according to any one of (1) to (20), wherein the cell is preferably a mammalian cell, more preferably a CHO cell and even more preferably a CHOK1SV;

(25) a method for producing an antibody or a functional fragment thereof according to any one of (1) to (20), comprising the following steps (I) and (II):

(I) culturing a cell according to (23) or (24); and

(II) collecting the antibody or the functional fragment thereof according to any one of (1) to (20) from the culture obtained in the step (I);

(26) an antibody or a functional fragment thereof produced by a production method according to (25);

(27) a modified form of an antibody or a functional fragment thereof according to any one of (1) to (20) and (26);

(28) a pharmaceutical composition comprising an antibody or a functional fragment thereof according to any one of (1) to (20) and (26) or a modified form according to (27) as an active ingredient;

(29) the pharmaceutical composition according to (28), wherein the pharmaceutical composition is an agent for treating or preventing an angiogenic disease;

(30) the pharmaceutical composition according to (28), wherein the angiogenic disease is exudative age-related macular degeneration, diabetic retinopathy, macular edema, benign or malignant tumor, atherosclerosis, retrolental fibroplasia, angioma, chronic inflammation, ocular neovascular disease, proliferative retinopathy, neovascular glaucoma, immune rejection of a corneal tissue transplant or other tissue transplants, rheumatoid arthritis, psoriasis, acute inflammation, sepsis, or obesity;

(31) the pharmaceutical composition according to (28), wherein the angiogenic disease is exudative age-related macular degeneration, diabetic retinopathy, macular edema, retrolental fibroplasia, ocular neovascular disease, proliferative retinopathy, neovascular glaucoma, or immune rejection of a corneal tissue transplant;

(32) an angiogenesis inhibitor comprising an antibody or a functional fragment thereof according to any one of (1) to (20) and (26) or a modified form according to (27) as an active ingredient;

(33) a method for treating or preventing an angiogenic disease comprising administering to a subject in need thereof an effective amont of an antibody or a functional fragment thereof according to any one of (1) to (20) and (26) or a modified form according to (27), or the composition according to any one of (28) to (31), preferably wherein the angiogenic disease is the angiogenic disease in an individual having the expressed ROBO4 protein; and

(34) the pharmaceutical composition according to any one of claims 28 to 31, wherein said composition is used in combination with a further therapeutic or prophylactic agent,

preferably wherein said agent is an anti-angiogenesis drug, anti-inflammatory drug, and/or an anticancer drug.

[Advantageous Effects of Invention]

According to the present invention, a therapeutic agent or the like for an angiogenic disease containing an antibody that binds to ROBO4 and has an anti-angiogenesis effect can be obtained.

[Brief Description of Drawings]

[Figure 1] Figure 1 is a diagram showing the presence or absence of change in reporter activity against NF-κB, GAS, ISRE, IL-8 promoter, or TCF as response elements in the transient expression of human ROBO4 in HEK293 cells. The error bar in the diagram represents standard deviation (n = 3).

[Figure 2] Figure 2 is a diagram showing the presence or absence of change in IL-8 promoter activity in the transient expression of full-length human ROBO4 or an intracellular region deletion variant of human ROBO4 in HEK293 cells. The error bar in the diagram represents standard deviation (n = 5 or 10).

[Figure 3] Figure 3 is a diagram showing change in IL-8 promoter activity in human ROBO4-transfected HEK293 cells caused by an anti-ROBO4 antibody MAb1. The error bar in the diagram represents standard deviation (n = 3).

[Figure 4] Figure 4 is a diagram showing change in IL-8 promoter activity in human ROBO4-transfected HEK293 cells caused by an anti-ROBO4 antibody MAb2, MAb3, or MAb4. The error bar in the diagram represents standard deviation (n = 3).

[Figure 5] Figure 5 is a diagram showing change in the migratory capacity of HUVEC in the presence of VEGF or bFGF caused by the anti-ROBO4 antibody MAb1. The error bar in the diagram represents standard deviation (n = 3 or 4).

[Figure 6] Figure 6 is a diagram showing change in the migratory capacity of HUVEC in the presence of bFGF caused by the anti-ROBO4 antibody MAb2, MAb3, or MAb4. The error bar in the diagram represents standard deviation (n = 4).

[Figure 7] Figure 7 is a diagram showing the presence or absence of the binding activity of the anti-ROBO4 antibody MAb1 against human ROBO4, mouse ROBO4, rat ROBO4, or cynomolgus monkey ROBO4.

[Figure 8] Figure 8 is a diagram showing the presence or absence of the binding activity of the anti-ROBO4 antibody MAb1 against human ROBO1, human ROBO2, or human ROBO3. The upper boxes show the results about MAb1, and the lower boxes show the results about a positive control antibody with which the expression of these proteins on cell surface was confirmed.

[Figure 9] Figure 9 is a diagram showing the presence or absence of the binding activity of the anti-ROBO4 antibody MAb1 against an extracellular region/domain deletion variant of human ROBO4. The upper boxes show the results about MAb1, and the lower boxes show the results about an anti-FLAG antibody with which the expression of these proteins on cell surface was confirmed.

[Figure 10] Figure 10 is a diagram showing change in angiogenesis in laser-induced choroidal neovascularization models caused by the anti-ROBO4 antibody MAb1. The error bar in the diagram represents standard deviation (n = 4 eyes), and ■ or □ shows the results of each eye.

[Figure 11] Figure 11 is a diagram showing change in IL-8 promoter activity in human ROBO4-transfected HEK293 cells caused by an anti-ROBO4 chimeric antibody cMAb1-1 or cMAb1-2. The error bar in the diagram represents standard deviation (n = 3).

[Figure 12] Figure 12 is a diagram showing change in the migratory capacity of HUVEC in the presence of bFGF caused by the anti-ROBO4 chimeric antibody

cMAb1-1 or cMAb1-2. The error bar in the diagram represents standard deviation (n = 4).

[Figure 13] Figure 13 shows cDNA encoding full-length human ROBO4 (SEQ ID NO: 1).

[Figure 14] Figure 14 shows the full-length amino acid sequence of human ROBO4 (SEQ ID NO: 2).

[Figure 15] Figure 15 shows the nucleotide sequence of cDNA encoding the heavy chain variable region of MAb1 (SEQ ID NO: 30).

[Figure 16] Figure 16 shows the amino acid sequence of the MAb1 heavy chain variable region (SEQ ID NO: 31).

[Figure 17] Figure 17 shows the nucleotide sequence of cDNA encoding the light chain variable region of MAb1 (SEQ ID NO: 32).

[Figure 18] Figure 18 shows the amino acid sequence of the MAb1 light chain variable region (SEQ ID NO: 33).

[Figure 19] Figure 19 shows the nucleotide sequence of cDNA encoding the light chain of cMAb1 (SEQ ID NO: 37).

[Figure 20] Figure 20 shows the amino acid sequence of the cMAb1 light chain (SEQ ID NO: 38).

[Figure 21] Figure 21 shows the nucleotide sequence of cDNA encoding the heavy chain of cMAb1-1 (SEQ ID NO: 39).

[Figure 22] Figure 22 shows the amino acid sequence of the cMAb1-1 heavy chain (SEQ ID NO: 40).

[Figure 23] Figure 23 shows the nucleotide sequence of cDNA encoding the heavy chain of cMAb1-2 (SEQ ID NO: 41).

[Figure 24] Figure 24 shows the amino acid sequence of the cMAb1-2 heavy chain (SEQ ID NO: 42).

[Figure 25] Figure 25 shows the amino acid sequence of the heavy chain CDRH1 of MAb1 (SEQ ID NO: 44).

[Figure 26] Figure 26 shows the amino acid sequence of the heavy chain CDRH2 of MAb1 (SEQ ID NO: 46).

[Figure 27] Figure 27 shows the amino acid sequence of the heavy chain CDRH3 of MAb1 (SEQ ID NO: 48).

[Figure 28] Figure 28 shows the amino acid sequence of the light chain CDRL1 of MAb1 (SEQ ID NO: 50).

[Figure 29] Figure 29 shows the amino acid sequence of the light chain CDRL2 of MAb1 (SEQ ID NO: 52).

[Figure 30] Figure 30 shows the amino acid sequence of the light chain CDRL3 of MAb1 (SEQ ID NO: 54).

[Figure 31] Figure 31 shows the nucleotide sequence of cDNA encoding a hMAb1-H1-type heavy chain (SEQ ID NO: 55).

[Figure 32] Figure 32 shows the amino acid sequence of the hMAb1-H1-type heavy chain (SEQ ID NO: 56).

[Figure 33] Figure 33 shows the nucleotide sequence of cDNA encoding a hMAb1-H2-type heavy chain (SEQ ID NO: 57).

[Figure 34] Figure 34 shows the amino acid sequence of the hMAb1-H2-type heavy chain (SEQ ID NO: 58).

[Figure 35] Figure 35 shows the nucleotide sequence of cDNA encoding a hMAb1-H3-type heavy chain (SEQ ID NO: 59).

[Figure 36] Figure 36 shows the amino acid sequence of the hMAb1-H3-type heavy chain (SEQ ID NO: 60).

[Figure 37] Figure 37 shows the nucleotide sequence of cDNA encoding a hMAb1-H4-type heavy chain (SEQ ID NO: 61).

[Figure 38] Figure 38 shows the amino acid sequence of the hMAb1-H4-type heavy chain (SEQ ID NO: 62).

[Figure 39] Figure 39 shows the nucleotide sequence of cDNA encoding a hMAb1-L1-type light chain (SEQ ID NO: 63).

[Figure 40] Figure 40 shows the amino acid sequence of the hMAb1-L1-type light chain (SEQ ID NO: 64).

[Figure 41] Figure 41 shows the nucleotide sequence of cDNA encoding a hMAb1-L2-type light chain (SEQ ID NO: 65).

[Figure 42] Figure 42 shows the amino acid sequence of the hMAb1-L2-type light chain (SEQ ID NO: 66).

[Figure 43] Figure 43 shows the amino acid sequence of CDRH1 of the hMAb1-H2- or hMAb1-H4-type heavy chain (SEQ ID NO: 67).

[Figure 44] Figure 44 shows the amino acid sequence of CDRH2 of the hMAb1-H2- or hMAb1-H4-type heavy chain (SEQ ID NO: 68).

[Figure 45] Figure 45 shows the amino acid sequence of CDRH3 of the hMAb1-H2- or hMAb1-H4-type heavy chain (SEQ ID NO: 69).

[Figure 46] Figure 46 shows the amino acid sequence of CDRL1 of the hMAb1-L2-type light chain (SEQ ID NO: 70).

[Figure 47] Figure 47 shows the amino acid sequence of CDRL2 of the hMAb1-L2-type light chain (SEQ ID NO: 71).

[Figure 48] Figure 48 shows the amino acid sequence of CDRL3 of the hMAb1-L2-type light chain (SEQ ID NO: 72).

[Figure 49] Figure 49 is a diagram showing change in IL-8 promoter activity in human ROBO4-transfected HEK293 cells caused by H-1040, H-1143, H-1140, H-2040, H-2143, or H-2140.

[Figure 50] Figure 50 is a diagram showing change in the migratory capacity of HUVEC in the presence of bFGF caused by H-1143, H-2140, or H-2143. The error bar in the diagram represents standard deviation (n = 4).

[Figure 51] Figure 51 is a diagram showing the cross-species reactivity of H-1143.

[Figure 52] Figure 52 is a diagram showing the cross-species reactivity of H-2140.

[Figure 53] Figure 53 is a diagram showing the cross-species reactivity of H-2143.

[Figure 54] Figure 54 is a diagram showing the binding specificity of H-1143, H-2140, or H-2143.

[Figure 55] Figure 55 is a diagram showing change in angiogenesis in laser-induced choroidal neovascularization models caused by H-2143. The error bar in the diagram represents standard deviation (n = 3-4 eyes).

[Mode for Carrying Out the Invention]

1. Definition

In the present invention, "gene" means nucleotide(s) or nucleotide sequence comprising a nucleotide sequence encoding the amino acids of a protein, or its complementary strand. The "gene" is meant to include, for example, a polynucleotide, an oligonucleotide, DNA, mRNA, cDNA, and cRNA as the nucleotide sequence comprising a nucleotide sequence encoding the amino acids of a protein, or its complementary strand. Such a gene is a single-stranded, double-stranded, or triple or more stranded nucleotide sequence, and the "gene" is also meant to include an associate of DNA and RNA strands, a mixture of ribonucleotides (RNAs) and deoxyribonucleotides (DNAs) on one nucleotide strand, and a double-stranded or triple or more stranded nucleotide sequence comprising such a nucleotide strand. Examples of the "ROBO4 gene" of the present invention can include DNA, mRNA, cDNA, and cRNA comprising a nucleotide sequence encoding the amino acid sequence of the ROBO4 protein.

In the present invention, the term "nucleotide(s)" or "nucleotide sequence" has the same meaning as in a "nucleic acid" and is also meant to include, for example, DNA, RNA, a probe, an oligonucleotide, a polynucleotide, and a primer. Such a nucleotide sequence is a single-stranded, double-stranded or triple or more stranded nucleotide, and the "nucleotide" sequence is also meant to include an associate of DNA and RNA strands, a mixture of ribonucleotides (RNAs) and deoxyribonucleotides (DNAs) on one nucleotide strand, and an associate of two strands or three or more strands comprising such a nucleotide strand.

In the present invention, the terms "polypeptide", "peptide", and "protein" have the same meaning.

In the present invention, the "antigen" is also used as the meaning of an "immunogen".

In the present invention, the "cell" also includes various cells derived from animal individuals, subcultured cells, primary cultured cells, cell lines, recombinant cells, and the like.

In the present invention, an antibody recognizing the ROBO4 protein is also referred to as an "anti-ROBO4 antibody". The "anti-ROBO4 antibody" includes an anti-ROBO4 chimeric antibody, an anti-ROBO4 humanized antibody, an anti-ROBO4 human antibody, and the like.

In the present invention, the "functional fragment of the antibody" means an antibody fragment that exerts at least one of the functions, e.g., the binding affinity (K_D value) of the original antibody. Examples of the "functional fragment of the antibody" can include, but not limited to, Fab, F(ab')2, scFv, Fab', single-chain immunoglobulin, and the like. Such a functional fragment of the antibody may be obtained by the treatment of a full-length molecule of the antibody protein with an enzyme such as papain or pepsin or may be a recombinant protein produced in an

appropriate host cell using a recombinant gene. Preferred "functional fragments" also have at least one of the biological activities of the original antibody.

Further, in the context of the present invention, a nucleotide sequence encoding "the partial amino acid sequence" of the heavy or light chain is or includes a nucleotide sequence encoding a "functional fragment of the antibody" as defined herein above.

In the present invention, the "site" to which an antibody binds, i.e., the "site" recognized by an antibody, means a partial peptide or partial conformation on an antigen bound or recognized by the antibody. In the present invention, such a site is also called an epitope or an antibody-binding site. Examples of the site on the ROBO4 protein bound or recognized by the anti-ROBO4 antibody of the present invention can include a partial peptide or partial conformation on the ROBO4 protein.

The heavy and light chains of an antibody molecule are known to each have three complementarity determining regions (CDRs). The complementarity determining regions are also called hypervariable domains. They are located in the variable regions of the antibody heavy and light chains. These sites have a particularly highly variable primary structure and are usually separated at three positions on the respective primary structures of heavy and light chain polypeptide strands. In the present invention, the complementarity determining regions of the antibody are referred to as CDRH1, CDRH2, and CDRH3 from the amino terminus of the heavy chain amino acid sequence as to the complementarity determining regions of the heavy chain and as CDRL1, CDRL2, and CDRL3 from the amino terminus of the light chain amino acid sequence as to the complementarity determining regions of the light chain. These sites are proximal to each other on the three-dimensional structure and determine specificity for the antigen to be bound.

In the present invention, the "antibody mutant" means a polypeptide that has an amino acid sequence derived from the amino acid sequence of the original antibody by the substitution, deletion, addition, and/or insertion (hereinafter, collectively referred to

as "mutation") of amino acid(s) and binds to the ROBO4 protein of the present invention. The number of the mutated amino acids in such an antibody mutant is 1 to 2, 1 to 3, 1 to 4, 1 to 5, 1 to 6, 1 to 7, 1 to 8, 1 to 9, 1 to 10, 1 to 12, 1 to 15, 1 to 20, 1 to 25, 1 to 30, 1 to 40, or 1 to 50. Such an antibody mutant is also encompassed by the "antibody" of the present invention.

In the present invention, the term "several" in "1 to several" refers to 2 to 10, preferably 2 to 8, more preferably 2.

Examples of activities or properties exerted by the antibody of the present invention can include biological activities or physicochemical properties and can specifically include various biological activities, a binding activity against an antigen or an epitope, stability during production or storage, and thermal stability.

In the present invention, the phrase "hybridizing under stringent conditions" means hybridization under conditions involving hybridization at 65°C in a solution containing 5 × SSC, followed by washing at 65°C for 20 minutes in an aqueous solution containing 2 × SSC-0.1% SDS, at 65°C for 20 minutes in an aqueous solution containing 0.5 × SSC-0.1% SDS, and at 65°C for 20 minutes in an aqueous solution containing 0.2 × SSC-0.1% SDS, or hybridization under conditions equivalent thereto. SSC means an aqueous solution of 150 mM NaCl-15 mM sodium citrate, and n × SSC means SSC with an n-fold concentration.

2. ROBO4 protein

In the present specification, the terms "ROBO4" and "ROBO4 protein" are used as the same meaning.

(2-1) Property

The ROBO4 protein of the present invention has the following properties:

(i) ROBO4 has a molecular weight of approximately 110 kDa and a single-pass transmembrane structure and is a receptor protein of the SLIT2 protein involved in

angiogenesis. Any ROBO4 protein of the present invention can be found in a form liberated from a membrane such as a cell membrane and may be in a form bound to a membrane such as a cell membrane. In this context, the molecular weight means an apparent molecular weight under the non-reducing conditions of SDS-PAGE. The N-terminal extracellular region of ROBO4 contains two immunoglobulin-like domains (hereinafter, referred to as "Ig-like domains") and two fibronectin type III domains, while its C-terminal intracellular region contains a protein-rich region. In the present specification, these two immunoglobulin-like domains are referred to as Ig-like domain 1 and Ig-like domain 2, respectively, from the amino terminus. The human ROBO4 protein consists of an amino acid sequence of amino acid Nos. 28 to 1007 of SEQ ID NO: 2 . Amino acid Nos. 1 to 27 of SEQ ID NO: 2 represent a secretory signal; amino acid Nos. 28 to 467 thereof represent an extracellular region; amino acid Nos. 46 to 131 thereof represent Ig-like domain 1; amino acid Nos. 137 to 224 thereof represent Ig-like domain 2; amino acid Nos. 252 to 340 thereof represent fibronectin type III domain 1; amino acid Nos. 347 to 438 thereof represent fibronectin type III domain 2; amino acid Nos. 468 to 490 thereof represent a region in the cell membrane; and amino acid Nos. 491 to 1007 thereof represent an intracellular region.

(ii) ROBO4 has an anti-angiogenesis effect. In the present invention, the term "anti-angiogenesis" means that the molecule directly or indirectly suppresses and/or inhibits angiogenesis by itself, in collaboration with another factor, or as an associate with another factor. The anti-angiogenesis effect can be evaluated, for example, with a suppressive effect on increase in vascular permeability, cell migration promoting activity, or lumen formation activity by VEGF as an index.

(iii) ROBO4 comprises an amino acid sequence described in any one of the following (a) to (e) (hereinafter, referred to as a "ROBO4 amino acid sequence"), consists of an amino acid sequence comprising the ROBO4 amino acid sequence, or consists of the ROBO4 amino acid sequence:

(a) the amino acid sequence represented by SEQ ID NO: 2 (Figure 14) ;

(b) the amino acid sequence of a polypeptide that exhibits 80% or more, 82% or more, 84% or more, 86% or more, 88% or more, 90% or more, 92% or more, 94% or more, 96% or more, 98% or more, or 99% or more sequence identity to the amino acid sequence represented by SEQ ID NO: 2 (Figure 14) and exhibits an anti-angiogenesis effect;

(c) the amino acid sequence of a polypeptide that comprises an amino acid sequence represented by SEQ ID NO: 2 (Figure 14) having a substitution, deletion, addition, or insertion of 1 to 50, 1 to 45, 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, 1 to 8, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 1 or 2, or 1 amino acid(s) and suppresses angiogenesis;

(d) the amino acid sequence of a polypeptide that comprises an amino acid sequence of SEQ ID NO: 2 having the deletion of amino acid Nos. 1 to 45 or 1 to 131 and suppresses angiogenesis; and

(e) the amino acid sequence of a polypeptide that is encoded by the nucleotide sequence of a nucleotide hybridizing under stringent conditions to a nucleotide having a nucleotide sequence complementary to a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 2 (Figure 14) and suppresses angiogenesis.

The ROBO4 protein may be present as the whole or a portion of a homo or hetero oligo associate constituted of two or more subunits.

The amino acid sequence and/or other properties of the ROBO4 protein may be neither the same nor homogeneous in an individual, a tissue, a body fluid, a cell, a ROBO4 protein-containing fraction, a purified or partially purified ROBO4 protein preparation, or the like, or among a plurality of individuals, tissues, cells, ROBO4 protein-containing fractions, or ROBO4 protein preparations. One individual, tissue, body fluid, cell, ROBO4 protein-containing fraction, purified or partially purified ROBO4 protein preparation, or the like may contain plural types of ROBO4 proteins differing in amino acid sequence and/or property. Alternatively, a plurality of

individuals, tissues, cells, ROBO4 protein-containing fractions, or ROBO4 protein preparations may differ in the amino acid sequence and/or other properties of the ROBO4 protein. Even such proteins differing in amino acid sequence and/or properties from each other are all encompassed by the "ROBO4 protein" of the present invention as long as they possess the properties described above in (i) to (iii).

(iv) The ROBO4 protein of the present invention can be obtained from the tissue of a vertebrate, preferably a mammal, more preferably a rodent such as a mouse or a rat or a human, even more preferably tissues of a human or a mouse, cells derived from such a tissue, cultures of such cells, and the like. Such a tissue and cells are not particularly limited as long as they contain the ROBO4 protein. Examples thereof can include joint tissues, blood, lymph, thymus glands, spleens, and cells derived from any of them. Preferable tissues and cells are tissues and cells derived from animals or patients having angiogenesis. However, the origin of the ROBO4 protein of the present invention is not limited to those described above, and the ROBO4 protein of the present invention is also meant to include even ROBO4 proteins derived from other animal species, other tissues, other cells, or the like as long as they possess the properties described above in (i) to (iii).

The ROBO4 protein of the present invention may be any of native and recombinant proteins. The ROBO4 protein is also meant to include fusion products with another peptide or protein such as a carrier or a tag. The ROBO4 protein is further meant to include forms provided with chemical modification including the addition of a polymer such as PEG and/or with biological modification including sugar chain modification. Moreover, the ROBO4 protein of the present invention is meant to include a ROBO4 protein fragment. A ROBO4 protein fragment possessing the property described above in (ii) is called a functional fragment of the ROBO4 protein.

(2-2) ROBO4 gene

The ROBO4 gene of the present invention comprises a nucleotide sequence described in any one of the following (a) to (c) (hereinafter, referred to as a "ROBO4 gene sequence"), consists of a nucleotide sequence comprising the ROBO4 gene sequence, or consists of the ROBO4 gene sequence:

(a) the nucleotide sequence represented by SEQ ID NO: 1 (Figure 13) ;

(b) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide consisting of a nucleotide sequence complementary to the nucleotide sequence represented by SEQ ID NO: 1 (Figure 13) and encodes the amino acid sequence of a polypeptide suppressing angiogenesis; and

(c) a nucleotide sequence that comprises a nucleotide sequence represented by SEQ ID NO: 1 (Figure 13) having a substitution, deletion, addition, or insertion of 1 to 150, 1 to 140, 1 to 130, 1 to 120, 1 to 110, 1 to 100, 1 to 90, 1 to 80, 1 to 70, 1 to 60, 1 to 50, 1 to 45, 1 to 40, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, 1 to 8, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 1 or 2, or 1 base(s) and encodes the amino acid sequence of a polypeptide suppressing angiogenesis.

The ROBO4 gene is overexpressed in blood vessels in fibrovascular membranes or intratumoral vessels of patients with a disease accompanied by angiogenesis, for example, proliferative diabetic retinopathy. In addition, the ROBO4 gene seems to be overexpressed in tissue or blood fractions derived from patients affected with a disease considered to involve angiogenesis, such as exudative age-related macular degeneration, macular edema, atherosclerosis, retrolental fibroplasia, angioma, chronic inflammation, ocular neovascular disease, proliferative retinopathy, neovascular glaucoma, immune rejection of a corneal tissue transplant or other tissue transplants, rheumatoid arthritis, psoriasis, acute inflammation, sepsis, or obesity, or from model animals of these diseases.

The expression and expression level of the ROBO4 gene may be assayed with any of a ROBO4 gene transcription product and the ROBO4 protein as an index and can

be determined by RT-PCR, Northern blot hybridization, or the like for the upper index and by immunoassay (e.g., enzyme-linked immuno-sorbent assay; hereinafter, referred to as "ELISA") or the like for the latter index.

(2-3) Preparation of protein

The ROBO4 protein of the present invention can be purified or isolated from animal tissues (including body fluids), cells derived from the tissues, or cultures of the cells and prepared by gene recombination, *in-vitro* translation, chemical synthesis, etc.

(2-3-1) Purification or isolation of native ROBO4

The native ROBO4 protein can be purified or isolated from, for example, tissues (including body fluids, cells, etc.) derived from patients or non-human animals affected with an angiogenic disease such as exudative age-related macular degeneration, diabetic retinopathy, macular edema, benign or malignant tumor, atherosclerosis, retrolental fibroplasia, angioma, chronic inflammation, ocular neovascular disease, proliferative retinopathy, neovascular glaucoma, immune rejection of a corneal tissue transplant or other tissue transplants, rheumatoid arthritis, psoriasis, acute inflammation, sepsis, or obesity, cells derived from the tissues, or cultures of the cells. Such non-human animals also include model animals of these diseases. Animals subjected to model preparation are not particularly limited as long as they are vertebrates. The animals are preferably mammals, more preferably rodents such as mice or rats, even more preferably mice or rats. The tissues and cells of such patients or model animals are not particularly limited as long as they contain the ROBO4 protein. Examples thereof can include joint tissues, blood, lymph, thymus glands, spleens, and cells derived from any of them. Preferable tissues and cells are derived from patients or model animals having angiogenesis or exhibiting similar symptoms. However, the origin of the ROBO4 protein of the present invention is not limited to those described above, and the

ROBO4 protein of the present invention may be derived from other animal species, other tissues, other cells, or the like.

Purification or isolation from such tissues, cells, cell cultures, or the like can be performed by the combination of approaches well known by those skilled in the art, such as fractionation and chromatography. Such approaches include, but not limited to, salting out, gel filtration, ion-exchange chromatography, affinity chromatography, hydrophobic chromatography, normal-phase or reverse-phase chromatography, and the like. An affinity gel cross-linked with an anti-ROBO4 monoclonal antibody can be prepared and loaded to a column to thereby prepare a column for affinity chromatography. A crude or partially purified fraction containing the ROBO4 protein is added to such a column. Subsequently, non-specific adsorbed matter is removed with sterilized phosphate-buffered saline (PBS), and a buffer solution for elution can then be added thereto to thereby selectively collect the ROBO4 protein. The solution containing the ROBO4 protein can be subjected to gel filtration or to buffer replacement and/or concentration using a concentrator such as Centriprep.

(2-3-2) Preparation of recombinant ROBO4 protein

The ROBO4 protein of the present invention can also be prepared in a recombinant form. Specifically, host cells are transfected with a gene encoding the amino acid sequence of the ROBO4 protein or a ROBO4 protein fragment, and the ROBO4 protein can be collected from cultures of the cells. For example, the ROBO4 gene or its fragment is inserted into an expression vector. Subsequently, prokaryotic or eukaryotic host cells are transfected with the resulting recombinant vector, and the obtained recombinant cells can be incubated to thereby express the ROBO4 protein. An expression pattern known in the art, such as secretion expression, intracellular expression of soluble forms, or an inclusion body method can be used. Also, the ROBO4 protein can be expressed not only as a molecule having the same amino

terminus (N-terminus) and/or carboxy terminus (C-terminus) as native ones, but also as a fusion protein with a secretory signal, an intracellular localization signal, a tag for affinity purification, or a partner peptide. The ROBO4 protein can be purified or isolated from such recombinant cell cultures by the appropriate combination of operations such as fractionation and chromatography described in (2-3-1) Purification or isolation of native ROBO4 protein.

The ROBO4 gene or its fragment can be prepared by a method well known by those skilled in the art.

Examples thereof can include: polymerase chain reaction (hereinafter, referred to as "PCR"; Saiki, R.K., et al., Science (1988) 239, p. 487-489) with a ROBO4 cDNA expression library as a template using one set of primers capable of specifically amplifying the sequence; reverse transcription PCR (hereinafter, referred to as "RT-PCR") with an mRNA fraction for ROBO4 expression as a template using a primer capable of reverse-transcribing the sequence and one set of primers capable of specifically amplifying the sequence; expression cloning using immunoassay; and cDNA cloning using the partial amino acid sequence of a purified ROBO4 protein.

(2-3-3) *In-vitro* translation

The ROBO4 protein of the present invention can also be prepared by *in-vitro* translation. Such a translation method is not particularly limited as long as it is a method using a cell-free translation system involving enzymes necessary for transcription and translation, substrates, and energy substances. Examples thereof can include a method using Rapid Translation System (RTS) manufactured by Roche Diagnostics.

(2-3-4) Chemical synthesis

The ROBO4 protein of the present invention can also be prepared by chemical synthesis. Examples of the chemical synthesis method can include solid-phase peptide synthesis methods such as Fmoc synthesis and Boc synthesis methods.

3. Anti-ROBO4 antibody

(3-1) Type of anti-ROBO4 antibody

The antibody of the present invention may be any of monoclonal and polyclonal antibodies. Examples of the monoclonal antibody of the present invention can include a non-human animal-derived antibody (non-human animal antibody), a human-derived antibody (human antibody), a chimeric antibody, and a humanized antibody, preferably a chimeric antibody, a humanized antibody, and a human-derived antibody (human antibody), more preferably a humanized antibody, and a human-derived antibody (human antibody).

Examples of the non-human animal antibody can include antibodies derived from vertebrates such as mammals and birds. Examples of the mammal-derived antibody can include rodent-derived antibodies such as mouse antibodies and rat antibodies. Examples of the bird-derived antibody can include chicken antibodies.

Examples of the chimeric antibody can include, but not limited to, an antibody comprising non-human animal antibody-derived variable regions bound with human antibody (human immunoglobulin) constant regions. Examples of the non-human animal antibody-derived variable regions can include heavy and light chain variable regions derived from MAb1 described later.

Examples of the humanized antibody can include, but are not limited to, a human antibody (human immunoglobulin variable regions) grafted with CDRs in the variable regions of a non-human animal antibody, a human antibody grafted with the CDRs as well as with partial sequences of framework regions of a non-human animal antibody, and an antibody having human antibody amino acid(s) replaced for one or two or more

non-human animal antibody-derived amino acid(s) in any of these humanized antibodies. Examples of the CDRs in the variable regions of a non-human animal antibody can include CDRH1 to 3 in the heavy chain variable region and CDRL1 to 3 in the light chain variable region derived from MAb1 described later.

The human antibody is not particularly limited as long as it is an antibody recognizing the antigen of the present invention. Examples thereof can include a human antibody binding to the same site as that bound by an antibody having the antibody CDRs of the present invention, and a human antibody binding to the same site on ROBO4 as that bound by MAb1 described above.

The antibody according to the present invention may be an antibody constituted of sites derived from a plurality of different antibodies. Examples thereof can include an antibody comprising heavy and/or light chains exchanged among a plurality of different antibodies, an antibody comprising full-length heavy and/or light chains exchanged thereamong, an antibody comprising variable or constant regions exchanged thereamong, and an antibody comprising all or some CDRs exchanged thereamong. The heavy and light chain variable regions of the chimeric antibody may be derived from different antibodies of the present invention. CDRH1 to 3 and CDRL1 to 3 in the heavy and light chain variable regions of the humanized antibody may be derived from two or more different antibodies of the present invention. CDRH1 to 3 and CDRL1 to 3 in the heavy and light chain variable regions of the human antibody may be the combination of CDRs carried by two or more different antibodies of the present invention.

The isotype of the monoclonal antibody of the present invention is not particularly limited, and examples thereof can include IgG such as IgG1, IgG2, IgG3, and IgG4, IgM, IgA such as IgA1 and IgA2, IgD, and IgE and can preferably include IgG and IgM, more preferably IgG2. The isotype and subclass of the monoclonal antibody can be determined by, for example, an Ouchterlony test, ELISA, radio

immunoassay (hereinafter, referred to as "RIA"). A commercially available kit for identification (Mouse Typer Kit manufactured by Bio-Rad Laboratories, Inc., RAT MONOCLONAL ANTIBODY ISOTYPING TEST KIT manufactured by AbD Serotec, etc.) may be used.

(3-2) Binding specificity of anti-ROBO4 antibody

The antibody of the present invention recognizes the ROBO4 protein. In other words, the antibody of the present invention binds to the ROBO4 protein. Such an antibody is referred to as an "anti-ROBO4 antibody". Moreover, the preferable antibody of the present invention specifically recognizes the ROBO4 protein. In other words, the preferable antibody of the present invention specifically binds to the ROBO4 protein. Furthermore, the more preferable antibody of the present invention specifically binds to an Ig-like domain carried by the ROBO4 protein. Examples of such an Ig-like domain can include Ig-like domain 1 and Ig-like domain 2. The more preferable antibody of the present invention recognizes a region consisting of an amino acid sequence of amino acid Nos. 132 to 209 of SEQ ID NO: 2 . The antibody of the present invention binds to a human ROBO4 protein, a monkey, preferably cynomolgus monkey ROBO4 protein, and a rabbit ROBO4 protein, but does not bind to mouse and rat ROBO4 proteins (Cross-species reactivity in Example 4)-3 and Example 11)-4).

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In the present invention, the "specific recognition", i.e., "specific binding", means binding which is not non-specific adsorption. Examples of criteria for determination on whether binding is specific or not can include a dissociation constant (hereinafter, referred to as "K_D"). The preferable antibody of the present invention has a K_D value of 1×10^{-5} or lower, 5×10^{-6} or lower, 2×10^{-6} or lower, or 1×10^{-6} or lower, more preferably 5×10^{-7} or lower, 2×10^{-7} or lower, or 1×10^{-7} or lower, even more preferably 5×10^{-8} or lower, 2×10^{-8} or lower, or 1×10^{-8} or lower, further more

preferably 5×10^{-9} or lower, 2×10^{-9} or lower, or 1×10^{-9} or lower, most preferably 5×10^{-10} or lower, 2×10^{-10} or lower, or 1×10^{-10} or lower for the ROBO4 protein. More specifically, the preferable antibody of the present invention has a K_D value of 2×10^{-8} or lower, more preferably 1×10^{-8} or lower, even more preferably 5×10^{-9} or lower for the ROBO4 protein.

In the present invention, the binding of the antibody to the antigen can be assayed or determined by ELISA, RIA, surface plasmon resonance (hereinafter, referred to as "SPR") analysis, or the like. Examples of equipment used in the SPR analysis can include BIAcore(TM) (manufactured by GE Healthcare Bio-Sciences Crop.), ProteOn(TM) (manufactured by Bio-Rad Laboratories, Inc.), SPR-Navi(TM) (manufactured by BioNavis Oy Ltd.), Spreeta(TM) (manufactured by Texas Instruments Inc.), SPRi-PlexII(TM) (manufactured by Horiba, Ltd.), and Autolab SPR(TM) (manufactured by Metrohm). The binding of the antibody to the antigen expressed on cell surface can be assayed by flow cytometry or the like.

(3-3) *In-vitro* anti-angiogenesis activity of anti-ROBO4 antibody

The antibody of the present invention has an anti-angiogenesis activity in the absence of a cross-linking antibody *in vitro*. It is known that certain antibodies do not exhibit a pharmacological activity in the absence of the cross-linking antibody *in vitro*, but exhibit a pharmacological activity in the absence of the cross-linking antibody *in vivo* (Cancer Cell (2011), 19, p. 101-113). This is probably because leukocytes are found *in vivo* to express Fcγ receptor having the same functions as those of the cross-linking antibody (Nature (2008), 8, p. 34-47); thus, the antibodies exhibit a pharmacological activity through crosslink in the presence of leukocytes even without the cross-linking antibody. In actual organisms, however, the number of leukocytes in lesions differs among individuals (Cancer Res (2011), 71, 5670-5677), presumably resulting in, among individuals, the different effects of the antibodies exhibiting a

pharmacological activity dependent on crosslink induced by leucocytes. The antibody of the present invention exhibits an excellent anti-angiogenesis activity even in the absence of a cross-linking antibody *in vitro*. Thus, the antibody of the present invention can also have an anti-angiogenesis effect independent of the number of leukocytes *in vivo* and is thus pharmaceutically suitable.

The anti-angiogenesis activity means the activity of suppressing vascular endothelial cell growth, migration, lumen formation, etc. The *in-vitro* anti-angiogenesis activity can be evaluated by a vascular permeability, vascular endothelial cell migration, or lumen formation test.

For example, the vascular permeability test for such evaluation can involve inoculating a normal human umbilical vein endothelial cell (HUVEC) to the upper layer of Boyden Chamber having a pore size of 1 μm to form a single layer and then measuring the amount of FITC-labeled dextran or the like permeating through the cell layer. The amount of FITC-labeled dextran permeating through the cell layer may be measured using, for example, In Vitro Vascular Permeability Assay (Cat. ECM640, manufactured by Millipore Corp.). When the antibody added at a concentration of 5 μg/mL or lower exhibits the effect of suppressing the amount of FITC-labeled dextran permeating through the cell layer, this antibody can be evaluated as having a suppressive effect on vascular permeability and having an anti-angiogenesis activity. The antibody of the present invention exhibits a suppressive activity against vascular permeability at a concentration of preferably 5 μg/mL or lower, more preferably 1 μg/mL or lower, particularly preferably 0.5 μg/mL or lower, under the measurement conditions described above.

The cell migration test for such evaluation can involve inoculating HUVEC to the upper layer of Boyden Chamber having a pore size of 3 to 8 μm, adding a medium containing an endothelial cell migration enhancer such as VEGF to the lower layer, and measuring the number of cells migrating to the lower layer. When the antibody

exhibits the effect of decreasing the number of migrating HUVEC cells, this antibody can be evaluated as having a suppressive effect on vascular endothelial cell migration and having an anti-angiogenesis activity. The number of migrating cells may be measured using, for example, vascular endothelial cell migration assay system (Cat. 354143, manufactured by BD Biosciences). The antibody of the present invention exhibits a suppressive activity against cell migration at a concentration of preferably 5μg/mL or lower, more preferably 1 μg/mL or lower, particularly preferably 0.5 μg/mL or lower, under the measurement conditions described above.

The lumen formation test for such evaluation can involve inoculating HUVEC to a cell culture container coated with Matrigel and measuring the number of branch points, tube length, or the like, of a lumen structure formed by HUVEC on the Matrigel. When the antibody exhibits the effect of decreasing the number of branch points or tube length of the lumen structure, this antibody can evaluated as having a suppressive effect on lumen formation and having an anti-angiogenesis activity. The number of branch points or tube length of the lumen structure may be measured using, for example, vascular endothelial cell tube formation assay system (Cat. 354149, manufactured by BD Biosciences). The antibody of the present invention exhibits a suppressive activity against lumen formation at a concentration of preferably 5 μg/mL or lower, more preferably 1 μg/mL or lower, particularly preferably 0.5 μg/mL or lower, under the measurement conditions described above.

Such an assay system, however, is not limited to these tests as long as it is capable of assaying angiogenesis and its suppression induced by the ROBO4 protein.

The cross-linking antibody means an antibody that binds to the Fc region of the antibody of the present invention and acts to cross-link two or more antibody molecules of the present invention. For example, when the Fc region of the antibody of the present invention is derived from a mouse, the cross-linking antibody refers to an antibody that binds to the mouse Fc region and associates two or more antibody

molecules of the present invention through the binding of these two antibody molecules of the present invention at two binding sites, respectively, of the cross-linking antibody.

The phrase "having an anti-angiogenesis activity in the absence of a cross-linking antibody" means that the antibody exhibits an anti-angiogenesis effect in an evaluation system relating to angiogenesis suppression, for example, the evaluation system described above, even without coexisting with a cross-linking antibody.

The phrase "having an anti-angiogenesis activity in the presence of a cross-linking antibody" means that the antibody does not exhibit an anti-angiogenesis activity in the absence of a cross-linking antibody in an evaluation system relating to angiogenesis, for example, the anti-angiogenesis activity evaluation system described above, but exhibits the anti-angiogenesis activity when coexisting with one or more cross-linking antibody molecule(s), preferably two or more cross-linking antibody molecules, with respect to one antibody molecule of the present invention.

(3-4) *In-vivo* suppressive or inhibitory activity of anti-ROBO4 antibody against angiogenesis

The antibody of the present invention suppresses or inhibits angiogenesis *in vivo*. The *in-vivo* suppressive or inhibitory activity against angiogenesis can be evaluated with animal disease models according to a standard method. For example, laser-induced choroidal neovascularization models described later in Example 4)-6 are widely used as disease models of angiogenesis and can be used in evaluation with the amount of blood vessels newly formed as a score. Also in the case of patients, for example, tumor samples are collected by biopsy from tumor patients before and after administration of the antibody of the present invention, and the vascular densities of their intratumoral vessels can be measured by immunohistochemical analysis (IHC) to score the amount of blood vessels newly formed.

(3-5) Activation of downstream signal by anti-ROBO4 antibody

The anti-ROBO4 antibody of the present invention may be subjected to an evaluation system using a cell line or primary cultured cells that exhibit some induced response to the ROBO4 protein. Examples of such a cell line can include a mouse vascular endothelial cell line (ATCC NO. CRL-2779). Examples of such primary cultured cells can include mouse vascular endothelial cells and human vascular endothelial cells.

The antibody of the present invention is an agonistic antibody against ROBO4. Specifically, the antibody of the present invention binds to ROBO4 and activates the downstream signal of ROBO4. Thus, the anti-angiogenesis effect of the antibody of the present invention may be evaluated with the activation of the ROBO4 downstream signal as an index. Examples of the ROBO4 downstream signal can include an IL-8 promoter activity. The IL-8 promoter activity was drastically increased in cells expressing full-length human ROBO4 compared with cells expressing no human ROBO4 and was hardly observed in cells expressing intracellular domain-deleted human ROBO4. Thus, the increase in IL-8 promoter activity demonstrated that the activation of the ROBO4 signal was detected (Example 3). The IL-8 promoter activity can be evaluated, for example, by the addition of the anti-ROBO4 antibody or the co-addition of the anti-ROBO4 antibody and a cross-linking antibody to cells transfected with a reporter vector having an IL-8 promoter sequence insert and a human ROBO4 expression plasmid, followed by the determination of the reporter activity.

(3-6) Anti-ROBO4 mouse monoclonal antibody and the chimeric antibody

MAb1 is an anti-ROBO4 mouse monoclonal antibody obtained by a method described in Example 2.

The nucleotide sequence of cDNA encoding the heavy chain variable region of MAb1 is shown in SEQ ID NO: 30 (Figure 15) , and its amino acid sequence is shown

in SEQ ID NO: 31 (Figure 16). The amino acid sequence of CDRH1 is shown in SEQ ID NO: 44 (Figure 25); the amino acid sequence of CDRH2 is shown in SEQ ID NO: 46 (Figure 26); and the amino acid sequence of CDRH3 is shown in SEQ ID NO: 48 (Figure 27). The nucleotide sequence of cDNA encoding the light chain variable region of MAb1 is shown in SEQ ID NO: 32 (Figure 17) , and its amino acid sequence is shown in SEQ ID NO: 33 (Figure 18). The amino acid sequence of CDRL1 is shown in SEQ ID NO: 50 (Figure 28); the amino acid sequence of CDRL2 is shown in SEQ ID NO: 52 (Figure 29); and the amino acid sequence of CDRL3 is shown in SEQ ID NO: 54 (Figure 30).

The antibody mutant of the present invention, preferably, exhibits reduced sensitivity to protein degradation or oxidation, an improved biological activity, an improved ability to bind to the antigen, or physicochemical or functional properties imparted thereto, or the like. Examples of such an antibody mutant can include an antibody having an amino acid sequence derived from the amino acid sequence of the original antibody by conservative amino acid substitution. The conservative amino acid substitution is substitution that occurs in an amino acid group related to amino acid side chains.

Preferable amino acid groups are as follows: an acidic group involving aspartic acid and glutamic acid; a basic group involving lysine, arginine, and histidine; a nonpolar group involving alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, and tryptophan; and an uncharged polar family involving glycine, asparagine, glutamine, cysteine, serine, threonine, and tyrosine. Other preferable amino acid groups are as follows: an aliphatic hydroxy group involving serine and threonine; an amide-containing group involving asparagine and glutamine; an aliphatic group involving alanine, valine, leucine, and isoleucine; and an aromatic group involving phenylalanine, tryptophan, and tyrosine. Such amino acid substitution in the

antibody mutant is preferably performed without reducing the antigen binding activity of the original antibody.

An antibody mutant having an amino acid sequence derived from the amino acid sequence of MAb1 of the present invention by conservative amino acid substitution as well as a mouse antibody, rat antibody, chimeric antibody, humanized antibody, human antibody, or the like comprising a CDR amino acid sequence derived from the amino acid sequence of any of MAb1-derived CDRH1 to 3 and CDRL1 to 3 by conservative amino acid mutation is also encompassed by the present invention.

The constant regions of the antibody of the present invention are not particularly limited. Preferably, those derived from a human antibody are used in the antibody of the present invention for the treatment or prevention of a disease in a human. Examples of the heavy chain constant region of the human antibody can include Cγ1, Cγ2, Cγ3, Cγ4, Cμ, Cδ, Cα1, Cα2, and Cε. Examples of the light chain constant region of the human antibody can include Cκ and Cλ.

A nucleotide sequence encoding the secretory signal-containing light chain of cMAb1-1 exemplified as the mouse-human IgG1-type chimeric antibody of the present invention and its amino acid sequence as well as a nucleotide sequence encoding the heavy chain thereof and its amino acid sequence are shown in SEQ ID NOs: 37, 38, 39, and 40 (Figures 19, 20, 21, and 22), respectively, . Likewise, a nucleotide sequence encoding the secretory signal-containing light chain of cMAb1-2 exemplified as the mouse-human IgG2-type chimeric antibody of the present invention and its amino acid sequence as well as a nucleotide sequence encoding the heavy chain thereof and its amino acid sequence are shown in SEQ ID NOs: 37, 38, 41, and 42 (Figures 19, 20, 23, and 24), respectively.

(3-7) Functional fragment of anti-ROBO4 antibody

According to one aspect, the present invention provides a functional fragment of the anti-ROBO4 antibody of the present invention. The functional fragment of the antibody means a fragment maintaining at least a portion of the functions of the antibody, or a modified form thereof described later in (3-10). Examples of such functions of the antibody can generally include an antigen binding activity, an antigen activity-regulating activity, an antibody-dependent cytotoxic activity, and a complement-dependent cytotoxic activity. Examples of the functions of the anti-ROBO4 antibody of the present invention can include a ROBO4 protein binding activity, an anti-angiogenesis activity, and a ROBO4 downstream signal-activating effect. More specifically, any functional fragment having all or some of the above-described activities (3-3) to (3-5) exhibited by the antibody against ROBO4 of the present invention is included in the functional fragment of the antibody of the present invention.

The functional fragment of the antibody is not particularly limited as long as it is a fragment of the antibody maintaining at least a portion of the activities of the antibody, or a modified form thereof. Examples thereof can include, but not limited to, Fab, F(ab')2, Fv, single-chain Fv (scFv) comprising heavy and light chain Fvs linked via an appropriate linker, diabodies, linear antibodies, polyspecific antibodies formed from antibody fragments, and Fab', which is a monovalent fragment of antibody variable regions obtained by the treatment of F(ab')2 under reducing conditions. A molecule containing a moiety other than the fragment of the antibody of the present invention, as in scFv carrying the linker moiety, is also encompassed in the meaning of the functional fragment of the antibody of the present invention.

A molecule that is derived from the antibody protein by the deletion of 1 to several or more amino acid(s) at its amino terminus and/or carboxy terminus and maintains at least a portion of the functions of the antibody is also encompassed in the meaning of the functional fragment of the antibody of the present invention.

The antibody of the present invention or the functional fragment thereof may be a polyspecific antibody having specificity for at least 2 types of different antigens. The polyspecific antibody is not limited to a bispecific antibody, which binds to 2 types of different antigens, and an antibody having specificity for 3 or more types of different antigens is also encompassed in the meaning of the "polyspecific antibody" of the present invention.

The polyspecific antibody of the present invention may be a full-length antibody or a functional fragment thereof (e.g., bispecific F(ab')2 antibody). The bispecific antibody can also be prepared by binding the heavy and light chains (HL pairs) of two types of antibodies. The bispecific antibody can also be obtained by fusing two or more types of monoclonal antibody-producing hybridomas to prepare bispecific antibody-producing fusion cells (Millstein et al., Nature (1983) 305, p. 537-539). The polyspecific antibody can also be prepared in the same way as above.

According to one aspect, the antibody of the present invention is a single-chain antibody (single-chain Fv; hereinafter, referred to as "scFv"). The scFv is obtained by linking the heavy and light chain variable regions of the antibody via a polypeptide linker (Pluckthun, The Pharmacology of Monoclonal Antibodies, 113, ed Rosenburg and Moore, Springer Verlag, New York, p.269-315 (1994), Nature Biotechnology (2005), 23, p.1126-1136). Moreover, bi-scFv comprising two scFvs linked via a polypeptide linker can be used as a bispecific antibody. Furthermore, multi-scFv comprising three or more scFvs can also be used as a polyspecific antibody.

The present invention includes a single-chain immunoglobulin comprising full-length heavy and light chain sequences of the antibody linked via an appropriate linker (Lee, H-S, et. al., Molecular Immunology (1999) 36, p.61-71; Shirrmann, T. et. al., mAbs (2010), 2, (1) p.1-4). Such a single-chain immunoglobulin can be dimerized to thereby maintain a structure and activities similar to those of the antibody, which is originally a tetramer. Also, the antibody of the present invention may be an antibody

that has a single heavy chain variable region and has no light chain sequence. Such an antibody is called a single domain antibody (sdAb) or a nanobody and has been reported to maintain the ability to bind to the antigen (Muyldemans S. et. al., Protein Eng. (1994) 7 (9), 1129-35, Hamers-Casterman C. et. al., Nature (1993) 363 (6428) 446-8). These antibodies are also encompassed in the meaning of the functional fragment of the antibody according to the present invention.

(3-8) Anti-human ROBO4 humanized antibody (hereinafter "anti-ROBO4 humanized antibody")

According to one aspect, the present invention provides a humanized antibody or a functional fragment thereof. The anti-ROBO4 humanized antibody of the present invention or the functional fragment thereof has an anti-angiogenesis activity and, preferably, has an anti-angiogenesis activity *in vivo*. Preferably, the humanized antibody or the functional fragment thereof specifically binds to the ROBO4 protein. Moreover, the humanized antibody or the functional fragment thereof is an agonistic antibody against ROBO4 and activates its downstream signal. Furthermore, the humanized antibody or the functional fragment thereof suppresses or inhibits vascular endothelial cell migration in the absence of a cross-linking antibody *in vitro*.

Examples of the humanized antibody of the present invention can include a human-derived antibody having MAb1 complementarity determining regions (CDRs) replaced with the CDRs of a non-human animal antibody (see Nature (1986) 321, p. 522-525), and a human antibody grafted with the CDR sequences and with some amino acid residues of framework regions by a CDR grafting method (International Publication No. WO90/07861). Furthermore, a variant derived from the humanized antibody by the substitution of 1 to 3 amino acid residues in each CDR with other amino acid residues is also included in the antibody of the present invention as long as the variant has all or some of the activities (3-3) to (3-5).

Preferred examples of the anti-ROBO4 humanized antibody of the present invention or the functional fragment thereof can include an antibody that consists of a heavy chain having a variable region comprising CDRH1 consisting of the amino acid sequence represented by SEQ ID NO: 44 (Figure 25) , CDRH2 consisting of the amino acid sequence represented by SEQ ID NO: 46 (Figure 26) or an amino acid sequence derived from the amino acid sequence of SEQ ID NO: 46 by the substitution of one amino acid, and CDRH3 consisting of the amino acid sequence represented by SEQ ID NO: 48 (Figure 27) , and a light chain having a variable region comprising CDRL1 consisting of the amino acid sequence represented by SEQ ID NO: 50 (Figure 28) or an amino acid sequence derived from the amino acid sequence of SEQ ID NO: 50 by the substitution of 1 to 3 amino acid(s), CDRL2 consisting of the amino acid sequence represented by SEQ ID NO: 52 (Figure 29) , and CDRL3 consisting of the amino acid sequence represented by SEQ ID NO: 54 (Figure 30) , and recognizes the ROBO4 protein of the present invention, and a fraction of the antibody maintaining the ROBO4 protein binding activity of the antibody.

Examples of the amino acid substitution in CDRH2 can include the substitution of the amino acid represented by amino acid No. 4 of SEQ ID NO: 46 in CDRH2. Specifically, an asparagine, amino acid No.4 of SEQ ID NO: 46 , can be replaced with a glutamine. The amino acid to be substituted therefor is not limited as long as the resulting antibody has all or some of the activities (3-3) to (3-5) exhibited by the antibody against ROBO4 of the present invention.

Examples of the amino acid substitution in CDRL1 can include the substitution of any 1 to 3, preferably 3, of the amino acids represented by amino acid Nos. 9, 11, and 13 of SEQ ID NO: 50 in CDRL1. Specifically, a serine (amino acid No.9), a glycine (amino acid No.11) and a threonine (amino acid No.13) of SEQ ID NO: 50 can be replaced with an amino acid selected from a glutamic acid, a lysine and a leucine,

preferably with a glutamic acid, a lysine and a leucine respectively. The amino acid(s) to be substituted therefor is not limited as long as the resulting antibody has all or some of the activities (3-3) to (3-5) exhibited by the antibody against ROBO4 of the present invention.

An asparagine residue in peptides or a protein is reported to easily undergo deamidation in some conditions (Gerger et al: The Journal of Biological Chemistry Vol.262 No.2, 785-794, 1987), therefore the amino acid replacement in CDRs described above can increase the stability of the humanized antibodies of the present invention.

Examples of the heavy chain variable region of the more preferred humanized antibody having these CDRHs can include an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 56 (Figure 32) , wherein CDRH1,CDRH2 and CDRH3 are represented by amino acid Nos. 50 to 54, 69 to 85 and 118 to 126 of SEQ ID NO: 56 (Figure 32), respectively, an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 58 (Figure 34) , wherein CDRH1,CDRH2 and CDRH3 are represented by amino acid Nos. 50 to 54, 69 to 85 and 118 to 126 of SEQ ID NO: 58 (Figure 34), respectively, an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 60 (Figure 36) , wherein CDRH1,CDRH2 and CDRH3 are represented by amino acid Nos. 50 to 54, 69 to 85 and 118 to 126 of SEQ ID NO: 60 (Figure 36), respectively, and an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 62 (Figure 38) , wherein CDRH1,CDRH2 and CDRH3 are represented by amino acid Nos. 50 to 54, 69 to 85 and 118 to 126 of SEQ ID NO: 60 (Figure 38). Examples of the light chain variable region of the more preferred humanized antibody having these CDRLs can include an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40) , wherein CDRL1,CDRL2 and CDRL3 are represented by amino acid Nos. 44 to 59, 75 to 81 and 114 to 122 of SEQ ID NO: 64 (Figure 40), respectively, and an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 66 (Figure 42) , wherein

CDRL1,CDRL2 and CDRL3 are represented by amino acid Nos. 44 to 59, 75 to 81 and 114 to 122 of SEQ ID NO: 66 (Figure 42), respectively.

Examples of more preferable combinations of the heavy chain variable region and the light chain variable region of the more preferred humanized antibodies can include: a humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 58 (Figure 34) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40) ; a humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 58 (Figure 34) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 66 (Figure 42) ; a humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 62 (Figure 38) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 66 (Figure 42) ; a humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 62 (Figure 38) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40) ; a humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 56 (Figure 32) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40) ; and a humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 60 (Figure 36) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40).

Even more preferred examples of the full-length humanized antibody comprising the more preferred combination of the heavy chain variable region and the light chain variable region can include: a humanized antibody (H-1140) comprising a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 58 (Figure 34) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 64 (Figure 40) ; a humanized antibody (H-1143) comprising a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 58 (Figure 34) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 66 (Figure 42) ; a humanized antibody (H-2143) comprising a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 62 (Figure 38) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 66 (Figure 42) ; a humanized antibody (H-2140) comprising a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 62 (Figure 38) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 64 (Figure 40) ; a humanized antibody (H-1040) comprising a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 56 (Figure 32) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 64 (Figure 40) ; and a humanized antibody (H-2040) comprising a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 60 (Figure 36) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 64 (Figure 40) .

The most preferable antibodies of the present invention are H-1140, H-1143, H-2140 and H-2143.

H-1140 has properties of 1) binding specifically to human ROBO4 and not to ROBO1, ROBO2, and ROBO3, 2)having a K_D value of 3.9nM for human ROBO4, 3) maintaining affinity to human ROBO4 under 40°C for 4weeks, 4) inhibiting HUVEC migration induced by one or more angiogenic factors selected from VEGF, bFGF, HGF, PDGF-BB and SDF-1, specifically VEGF or bFGF, 5) inhibiting angiogenesis in vivo, and 6) low immunogenicity in ISPRI Web-based Immunogenicity Screening (EpiVax, Inc).

H-1143 has properties of 1) binding specifically to human ROBO4 and not to ROBO1, ROBO2, and ROBO3, 2)having a K_D value of 3.5nM for human ROBO4, 3) maintaining affinity to human ROBO4 under 40°C for 4weeks, 4) inhibiting HUVEC migration induced by one or more angiogenic factors selected from VEGF, bFGF, HGF, PDGF-BB and SDF-1, specifically VEGF and bFGF, 5) inhibiting angiogenesis in vivo, and 6) low immunogenicity in EpiScreen™ immunogenicity testing (Antitope Ltd.)

H-2140 has properties of 1) binding specifically to human ROBO4 and not to ROBO1, ROBO2, and ROBO3, 2) having a K_D value of 1.8nM for human ROBO4, 3) maintaining affinity to human ROBO4 under 40°C for 4weeks, 4) inhibiting HUVEC migration induced by various angiogenic factors such as VEGF, bFGF, HGF, PDGF-BB and SDF-1, specifically VEGF and bFGF, 5) inhibiting angiogenesis in vivo, 6) low immunogenicity in EpiScreen™ immunogenicity testing (Antitope Ltd.)

H-2143 has properties of 1) binding specifically to human ROBO4 and not to ROBO1, ROBO2, and ROBO3, 2) having a K_D value of 1.7nM for human ROBO4, 3) maintaining affinity to human ROBO4 under 40°C for 4weeks, 4) inhibiting HUVEC migration induced by various angiogenic factors such as VEGF, bFGF, HGF, PDGF-BB and SDF-1, specifically VEGF and bFGF, 5) inhibiting angiogenesis in vivo, 6) low immunogenicity in EpiScreen™ immunogenicity testing (Antitope Ltd.), and 7) showing no serious change in clinical sign, body weight, food consumption,

hematology, blood chemistry, pathology, electroretinography after single intravitreal injection (2.75mg/eye) to a Cynomolgus monkey.

An antibody comprising an amino acid sequence having 95% or more, preferably 97% or more, more preferably 99% or more identity to the amino acid sequence of the antibody such as H-1140, H-1143, H-2143, H-2140, H-1040 and H-2040 is also included in the antibody of the present invention as long as the antibody has all or some of the activities (3-3) to (3-5). Moreover, an antibody that has CDRs identical in amino acid sequence to the CDRs of the antibody comprising the combination of the heavy chain variable region and the light chain variable region or the antibody comprising the combination of the heavy chain and the light chain, and has an amino acid sequence other than the CDR amino acid sequence having 95% or more, preferably 97% or more, more preferably 99% or more identity thereto is also included in the antibody of the present invention as long as the antibody has all or some of the activities (3-3) to (3-5).

(3-9) Antibody binding to the same site

An "antibody binding to the same site" as that bound by the antibody provided by the present invention is also included in the antibody of the present invention. The "antibody binding to the same site" as that bound by a certain antibody means another antibody that binds to a site on an antigen molecule recognized by the antibody. If a second antibody binds to a partial peptide or a partial three-dimensional structure on an antigen molecule bound by a first antibody, the first and second antibodies can be determined to bind to the same site. Moreover, the first and second antibodies can be determined to bind to the same site by confirming that the second antibody competes with the first antibody for binding to the antigen, i.e., the second antibody interferes with the binding of the first antibody to the antigen, even if the peptide sequence or three-dimensional structure of the specific binding site is not determined. Furthermore,

when the first and second antibodies bind to the same site and the first antibody has an effect characteristic of one aspect of the antibody of the present invention, such as an anti-angiogenesis activity, the second antibody also has an exceedingly high probability of having the same activity thereas. Thus, if a second anti-ROBO4 antibody binds to the site bound by a first anti-ROBO4 antibody, the first and second antibodies can be determined to bind to the same site on the ROBO4 protein. Moreover, the first and second antibodies can be determined to be antibodies binding to the same site on the ROBO4 protein by confirming that the second anti-ROBO4 antibody competes with the first anti-ROBO4 antibody for binding to the ROBO4 protein.

An antibody binding to a site on the ROBO4 protein recognized by MAb1 of the present invention is also included in the present invention.

The antibody-binding site can be determined by a method well known by those skilled in the art, such as immunoassay. For example, a series of peptides are prepared by the appropriate C-terminal or N-terminal truncation of the amino acid sequence of the antigen, and the reactivity of the antibody thereto is studied to roughly determine a recognition site. Then, shorter peptides are synthesized, and the reactivity of the antibody to these peptides can be studied to thereby determine the binding site. The antigen fragment peptides can be prepared using a technique such as gene recombination or peptide synthesis.

When the antibody binds to or recognizes the partial conformation of the antigen, the binding site for the antibody can be determined by identifying amino acid residues on the antigen adjacent to the antibody using an X-ray structural analysis.

(3-10) Modified form of anti-ROBO4 antibody or functional fragment thereof

The present invention provides a modified form of the antibody or the functional fragment thereof. The modified form of the antibody of the present invention or the functional fragment thereof means an antibody of the present invention or a functional

fragment thereof provided with chemical or biological modification. The chemically modified form includes a form having an amino acid skeleton conjugated with a chemical moiety, a form having a chemically modified N-linked or O-linked carbohydrate chain, and the like. Said chemical moiety or form can be toxic or cytotoxic.

The biologically modified form includes a form that has undergone post-translational modification (e.g., N-linked or O-linked glycosylation, N-terminal or C-terminal processing, deamidation, isomerization of aspartic acid, or oxidation of methionine), a form containing a methionine residue added to the N-terminus by expression using prokaryotic host cells, and the like. Such a modified form is also meant to include a form labeled to permit detection or isolation of the antibody or the antigen of the present invention, for example, an enzyme-labeled form, a fluorescently labeled form, or an affinity-labeled form. Such a modified form of the antibody of the present invention or the functional fragment thereof is useful in improvement in the stability or blood retention of the original antibody of the present invention or functional fragment thereof, reduction in antigenicity, detection or isolation of the antibody or the antigen, etc.

Examples of the chemical moiety contained in the chemically modified form can include water-soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, and polyvinyl alcohol.

Examples of the biologically modified form can include a form modified by enzymatic treatment, cell treatment, or the like, a form fused with another peptide, such as a tag, added by gene recombination, and a form prepared from host cells expressing an endogenous or exogenous sugar chain-modifying enzyme.

Such a modification may be made at an arbitrary position or the desired position in the antibody or the functional fragment thereof. Alternatively, the same or two or more different modifications may be made at one or two or more positions therein.

In the present invention, the "modified form of the antibody fragment" is also meant to include even a "fragment of the modified form of the antibody".

For example, occasionally, an antibody produced by cultured mammalian cells is known to lack a carboxyl-terminal lysine residue in its heavy chain (Journal of Chromatography A, 705: 129-134 (1995)). It is also known that occasionally 2 carboxyl-terminal amino acid residues (i.e., glycine and lysine) of a heavy chain are missing and that a proline residue newly located at the carboxyl terminus is amidated (Analytical Biochemistry, 360: 75-83 (2007)). Such lack or modification in these heavy chain sequences, however, affects neither the ability of the antibody to bind to its antigen nor the effector functions (complement activation, antibody-dependent cytotoxicity, etc.) of the antibody. Thus, an antibody having the modification and a functional fragment of the antibody are also included in the antibody of the present invention. Examples of such an antibody can include a deletion mutant derived from the antibody of the present invention by the deletion or lack of 1 or 2 amino acid(s) in the carboxyl terminus of the heavy chain, and the deletion mutant having an amidated residue (e.g., an amidated proline residue at the carboxyl-terminal site of the heavy chain). However, the deletion mutant ofthe antibody according to the present invention is not limited to the types described above as long as the deletion mutant maintains the ability to bind to the antigen and all or some of the activities (3-3) to (3-5). Two heavy chains constituting the antibody according to the present invention may be composed of any one type of heavy chain selected from the group consisting of the full-length heavy chains and the heavy chains of the deletion mutant or may be composed of the combination of any two types selected therefrom. The quantitative ratio of the deletion variant heavy chain(s) is susceptible to, for example, the type of cultured mammalian cells producing the antibody according to the present invention, and the culture conditions of the cells. Examples of such deletion variant heavy chains as the main components of the antibody according to the present invention can include two heavy chains, both of which lack one carboxyl-terminal amino acid residue. All of

these deletion variants are encompassed in the antibody variant, the functional fragment of the antibody, or the modified form thereof according to the present invention.

4. Method for producing antibody

(4-1) Method using hybridoma

In order to prepare the anti-ROBO4 antibody of the present invention, anti-ROBO4 antibody-producing cells are isolated from the spleens of animals immunized with the ROBO4 protein according to the method of Kohler and Milstein (Kohler and Milstein, Nature (1975) 256, p.495-497, Kennet, R. ed., Monoclonal Antibody, p.365-367, Prenum Press, N. Y. (1980)). The cells are fused with myeloma cells to thereby establish hybridomas, and monoclonal antibodies can be obtained from cultures of these hybridomas.

(4-1-1) Preparation of antigen

The antigen for the preparation of the anti-ROBO4 antibody can be obtained according to, for example, a native or recombinant ROBO4 protein preparation method described in other paragraphs of the present specification. Examples of the antigen that may be thus prepared can include the ROBO4 protein or a ROBO4 protein fragment comprising a partial sequence with at least 6 consecutive amino acids thereof, and their derivatives further comprising an arbitrary amino acid sequence or carrier added thereto (hereinafter, collectively referred to as a "ROBO4 antigen").

The recombinant ROBO4 antigen can be prepared by transfecting host cells with a gene comprising a nucleotide sequence encoding the amino acid sequence of the ROBO4 antigen, and collecting the antigen from cultures of the cells. The native ROBO4 antigen can be purified or isolated from, for example, human or rodent tissues with angiogenesis, cells derived from the tissues, or cultures of the cells. A ROBO4 antigen obtained in a cell-free *in-vitro* translation system from a gene comprising a

nucleotide sequence encoding the amino acid sequence of the ROBO4 antigen is also included in the "ROBO4 antigen" of the present invention.

(4-1-2) Production of anti-ROBO4 monoclonal antibody

The monoclonal antibody is typically produced by the following steps:

(a) preparing an antigen,

(b) preparing antibody-producing cells,

(c) preparing myeloma cells (hereinafter, referred to as "myelomas"),

(d) fusing the antibody-producing cells with the myelomas,

(e) screening for a hybridoma group producing the antibody of interest, and

(f) obtaining single cell clones (cloning).

This production method further involves (g) a step of culturing the hybridomas, a step of raising hybridoma-transplanted animals, etc., (h) a step of assaying or determining the biological activity of the monoclonal antibody, etc., if necessary.

Hereinafter, the method for preparing the monoclonal antibody will be described in detail with reference to these steps. However, the method for preparing the antibody is not limited to them, and, for example, antibody-producing cells other than spleen cells and myelomas may be used.

(a) Step of preparing antigen

An antigen can be prepared according to the ROBO4 protein preparation method described above in (2-3).

(b) Step of preparing antibody-producing cells

The antigen obtained in step (a) is mixed with an adjuvant such as a Freund's complete or incomplete adjuvant or potassium aluminum sulfate, and laboratory animals are immunized with the resulting immunogen. Any laboratory animal used in a hybridoma preparation method known in the art can be used without limitations. Specifically, for example, mice, rats, goats, sheep, cattle, or horses can be used. From

the viewpoint of readily available myeloma cells to be fused with isolated antibody-producing cells, etc., the animals to be immunized are preferably mice or rats.

Mouse and rat strains actually used are not particularly limited. In the case of mice, for example, A, AKR, BALB/c, BALB/cAnNCrj, BDP, BA, CE, C3H, 57BL, C57BL, C57L, DBA, FL, HTH, HT1, LP, NZB, NZW, RF, R III, SJL, SWR, WB, 129 can be used. In the case of rats, for example, Wistar, Low, Lewis, Sprague-Dawley, ACI, BN, or Fischer can be used.

These mice and rats can be available from laboratory animal breeders/distributors, for example, CLEA Japan, Inc. or Charles River Laboratories Japan Inc.

Of them, a BALB/c mouse strain or Wistar and Low rat strains are particularly preferable as animals to be immunized in consideration of fusion compatibility with the myeloma cells described later.

Also in consideration of the homology between human and mouse antigens, mice whose biological mechanism to remove autoantibodies has been reduced, i.e., autoimmune disease mice, is also preferably used.

In this context, these mice or rats are preferably 5 to 12 weeks old, more preferably 6 to 8 weeks old at the time of immunization.

The animals can be immunized with the ROBO4 protein using, for example, the method of Weir, D. M., Handbook of Experimental Immunology Vol. I. II. III., Blackwell Scientific Publications, Oxford (1987), Kabat, E. A. and Mayer, M. M., Experimental Immunochemistry, Charles C Thomas Publisher Spigfield, Illinois (1964).

Examples of antibody titer determination methods can include, but not limited to, immunoassay such as RIA and ELISA.

Antibody-producing cells derived from spleen cells or lymphocytes separated from the immunized animals can be prepared according to a method known in the art, for example, the method of Kohler et al., Nature (1975) 256, p.495,; Kohler et al., Eur. J.

Immnol. (1977) 6, p.511,; Milstein et al., Nature (1977), 266, p.550,; Walsh, Nature, (1977) 266, p.495,).

In the case of spleen cells, a general method can be adopted, which involves chopping the spleens, filtering cells through a stainless mesh, and then floating the resulting cells in an Eagle's minimum essential medium (MEM) or the like to separate antibody-producing cells.

(c) Step of preparing myelomas

The myeloma cells used in cell fusion are not particularly limited and can be selected appropriately for use from cell lines known in the art. An HGPRT (hypoxanthine-guanine phosphoribosyl transferase)-deficient line, i.e., mouse-derived X63-Ag8 (X63), NS1-ANS/1 (NS1), P3X63-Ag8.Ul (P3Ul), X63-Ag8.653 (X63.653), SP2/0-Ag14 (SP2/0), MPC11-45.6TG1.7 (45.6TG), FO, S149/5XXO, BU.1 or the like, rat-derived 210.RSY3.Ag.1.2.3 (Y3) or the like, or human-derived U266AR (SKO-007), GM1500-GTG-A12 (GM1500), UC729-6, LICR-LOW-HMy2 (HMy2), 8226AR/NIP4-1 (NP41), or the like, whose screening procedures have already been established, is preferably used in consideration of convenience in the selection of hybridomas from fusion cells. These HGPRT-deficient lines can be available from, for example, American Type Culture Collection (ATCC).

These cell lines are subcultured in an appropriate medium, for example, an 8-azaguanine medium [RPMI-1640 medium supplemented with glutamine, 2-mercaptoethanol, gentamicin, and fetal calf serum (hereinafter, referred to as "FCS") and further supplemented with 8-azaguanine], an Iscove's modified Dulbecco's medium (hereinafter, referred to as "IMDM"), or a Dulbecco's modified Eagle medium (hereinafter, referred to as "DMEM") and subcultured 3 to 4 days before cell fusion in a normal medium [e.g., ASF104 medium (manufactured by Ajinomoto Co., Inc.) containing 10% FCS] to secure the number of cells equal to or larger than 2×10^7 cells on the day of cell fusion.

(d) Step of fusing the antibody-producing cells with the myeloma cells

The antibody-producing cells can be fused with the myeloma cells under conditions that prevent cell viability from being exceedingly reduced, according to a method known in the art (Weir, D. M., Handbook of Experimental Immunology Vol. I. II. III., Blackwell Scientific Publications, Oxford (1987), Kabat, E. A. and Mayer, M. M., Experimental Immunochemistry, Charles C Thomas Publisher Spigfield, Illinois (1964) etc.). For example, a chemical method which involves mixing antibody-producing cells with myeloma cells in a high-concentration solution of a polymer such as polyethylene glycol, or a physical method using electric stimulation can be used.

(e) Step of screening for a hybridoma group producing the antibody of interest

A selection method for the hybridomas obtained by cell fusion is not particularly limited, and HAT (hypoxanthine-aminopterin-thymidine) selection method (Kohler et al., Nature (1975) 256, p.495; Milstein et al., Nature (1977) 266, p.550) is typically used. This method is effective for obtaining hybridomas using an HGPRT-deficient myeloma cell line, which cannot survive in the presence of aminopterin. Specifically, unfused cells and hybridomas can be cultured in a HAT medium to thereby allow only hybridomas resistant to aminopterin to selectively remain and grow.

(f) Step of obtaining single cell clones (cloning)

The hybridomas can be cloned using a method known in the art, for example, a methylcellulose, soft agarose, or limiting dilution method (see e.g., Barbara, B. M. and Stanley, M. S.: Selected Methods in Cellular Immunology, W. H. Freeman and Company, San Francisco (1980)). The limiting dilution method is preferable.

(g) Step of culturing the hybridomas and step of raising hybridoma-transplanted animals

The selected hybridomas can be cultured to thereby produce monoclonal antibodies. Preferably, the desired hybridomas are cloned and then subjected to antibody production.

The monoclonal antibody produced by this hybridoma can be collected from cultures of the hybridoma. Moreover, a recombinant antibody can also be collected from cultures of cells transfected with the monoclonal antibody gene. Furthermore, the hybridomas can be injected intraperitoneally to mice of the same strain (e.g., BALB/cAnNCrj described above) or Nu/Nu mice and allowed to grow. Then, the monoclonal antibody can also be collected from their ascites.

(h) Step of assaying or determining the biological activity of the monoclonal antibody

Various biological tests can be selected and applied thereto according to the purpose.

(4-2) Cell immunization method

Cells expressing the native ROBO4 protein, cells expressing the recombinant ROBO4 protein or its fragment, or the like can be used as immunogens to thereby prepare an anti-ROBO4 antibody by the hybridoma method described above.

Examples of the cells expressing the native ROBO4 protein can include cells derived from patients affected with an angiogenic disease such as proliferative diabetic retinopathy or tumor, and cells derived from the tissues of these patients. Such cells are preferably vascular endothelial cells, but not limited to them. These ROBO4 protein-expressing cells are used in an amount of 1×10^5 to 1×10^9 cells, preferably 1×10^6 to 1×10^8 cells, more preferably 0.5 to 2×10^7 cells, even more preferably 1×10^7 cells, in one immunization. The number of cells subjected to immunization can be changed according to the expression level of the ROBO4 protein. The immunogens are generally administered intraperitoneally and may be administered through an intradermal route or the like. The method described in (4-1-2) can be applied to the hybridoma preparation approach.

(4-3) Gene recombination and host cells

In order to prepare the antibody of the present invention, host cells are transfected with a nucleotide comprising a nucleotide sequence encoding the amino acid sequence of its heavy chain (heavy chain nucleotide) and a nucleotide comprising a nucleotide sequence encoding the amino acid sequence of its light chain (light chain nucleotide), or with a vector containing an insert of the heavy chain nucleotide and a vector containing an insert of the light chain nucleotide, and then cultured, and the antibody can be collected from the cultures. The heavy and light chain nucleotides may be inserted in one vector.

Prokaryotic or eukaryotic cells can be used as host cells. When eukaryotic cells are used as hosts, animal cells, plant cells, or eukaryotic microbes can be used.

Examples of the animal cells can include mammal-derived cells, i.e., monkey-derived COS cells (Gluzman, Y. Cell (1981) 23, p. 175-182, ATCC CRL-1650), mouse fibroblast NIH3T3 (ATCC No.CRL-1658), mouse NS0 cell lines (ECACC), Chinese hamster ovary cells (CHO cells, ATCC CCL-61), dihydrofolate reductase-deficient lines thereof (CHOdhfr-: Urlaub, G. and Chasin, L. A. Proc. Natl. Acad. Sci. U. S. A. (1980) 77, p.4126-4220), CHOK1SV developed by Lonza Biologics, cells derived from birds such as chickens, and cells derived from insects.

Also, host cells of the present invention include cells that may produce an antibody protein, wherein the structure of a sugar chain attached to the antibody protein is modified, wherein a biological activity of the antibody with the modification is preferably enhanced compared to the antibody without the modification. Example of such host cells of the present invention includes CHO cells that may produce an antibody protein having complex N-glycoside-linked sugar chains bound to the Fc region of the antibody, wherein among the total complex N-glycoside-linked sugar chains bound to the Fc region of the antibody, the ratio of a sugar chain in which fucose is not bound to N-acetylglucosamine in the reducing end in the sugar chain is 20% or more (WO2000/61739, WO2002/31140).

Examples of the eukaryotic microbes can include yeasts. Examples of the prokaryotic cells can include *E. coli* and *Bacillus subtilis*.

Mammal-derived cells are preferably used, CHO cells are more preferably used, and CHOK1SV are even more preferably used as host cells for producing the anti-ROBO4 antibodies of the present invention.

A signal peptide for the secretion of the antibody of the present invention (monoclonal antibodies derived from various animal species, rat antibody, mouse antibody, chimeric antibody, humanized antibody, human antibody, etc.) is not limited to the secretory signal of an antibody of the same species, the same type, and the same subtype as the antibody or to the secretory signal of the antibody itself. Any secretory signal of an antibody of different type or subtype therefrom or any secretory signal of a protein derived from a different eukaryotic species therefrom or a prokaryotic species can be selected and used.

(4-4) Methods for designing and preparing a humanized antibody

Examples of the humanized antibody can include, but not limited to, a human-derived antibody having CDRs replaced with the CDRs of a non-human animal antibody (see Nature (1986) 321, p. 522-525), a human antibody grafted with the CDR sequences and with some amino acid residues of framework regions by a CDR grafting method (see WO90/07861 and US6972323), and an antibody having human antibody amino acid(s) replaced for one or two or more non-human animal antibody-derived amino acid(s) in any of these humanized antibodies.

(4-5) Method for preparing a human antibody

Further examples of the antibody of the present invention can include a human antibody. The anti-ROBO4 human antibody means an anti-ROBO4 antibody consisting of the amino acid sequence of a human-derived antibody. The anti-ROBO4

human antibody can be obtained by a method using human antibody-producing mice carrying a human genomic DNA fragment comprising human antibody heavy chain- and light chain-encoding genes (see Tomizuka, K. et al., Nature Genetics (1997) 16, p.133-143,; Kuroiwa, Y. et. al., Nuc. Acids Res. (1998) 26, p.3447-3448; Yoshida, H. et. al., Animal Cell Technology: Basic and Applied Aspects vol. 10, p.69-73 (Kitagawa, Y., Matuda, T. and Iijima, S. eds.), Kluwer Academic Publishers, 1999.; Tomizuka, K. et. al., Proc. Natl. Acad. Sci. USA (2000) 97, p.722-727 etc.).

Specifically, such human antibody-producing animals can be prepared by disrupting the endogenous immunoglobulin heavy and light chain gene loci of non-human mammals and instead introducing thereto human immunoglobulin heavy and light chain gene loci via yeast artificial chromosome (YAC) vectors or the like. Alternatively, eukaryotic cells are transformed with heavy chain- and light chain-encoding cDNAs of such a human antibody, preferably with vectors comprising each of the cDNAs, by a gene recombination technique, and the transformed cells producing a recombinant human monoclonal antibody are cultured. This antibody can be obtained from the culture supernatant.

In this context, for example, eukaryotic cells, preferably mammalian cells such as CHO cells, lymphocytes, or myelomas can be used as hosts.

Also, a method for obtaining a phage display-derived human antibody selected from a human antibody library (see Wormstone, I. M. et. al, Investigative Ophthalmology & Visual Science. (2002) 43 (7), p.2301-2308; Carmen, S. et. al., Briefings in Functional Genomics and Proteomics (2002), 1 (2), p.189-203; Siriwardena, D. et. al., Opthalmology (2002) 109 (3), p.427-431 etc.) is known.

For example, a phage display method (Nature Biotechnology (2005), 23, (9), p. 1105-1116) can be used, which involves allowing the variable regions of a human antibody to be expressed as a single-chain antibody (scFv) on phage surface and selecting a phage binding to the antigen.

The phage selected based on its binding to the antigen can be subjected to gene analysis to thereby determine DNA sequences encoding the variable regions of the human antibody binding to the antigen.

If the DNA sequence of scFv binding to the antigen is determined, an expression vector having this sequence is prepared and appropriate hosts can be transfected with the expression vector and allowed to express the human antibody (WO92/01047, WO92/20791, WO93/06213, WO93/11236, WO93/19172, WO95/01438, WO95/15388, Annu. Rev. Immunol (1994) 12, p.433-455, Nature Biotechnology (2005) 23 (9), p.1105-1116.

(4-6) Method for preparing functional fragments of an antibody

The method for preparing a single-chain antibody is well known in the art (see e.g., US. Patent Nos. 4,946,778, 5,260,203, 5,091,513, and 5,455,030). In this scFv, a heavy chain variable region and a light chain variable region are linked via a linker that prevents them from forming a conjugate, preferably a polypeptide linker (Huston, J.S. et al., Proc. Natl. Acad. Sci. U.S.A. (1988), 85, p. 5879-5883). The heavy chain variable region and the light chain variable region in scFv may be derived from the same antibody or may be derived from different antibodies.

For example, an arbitrary single-chain peptide consisting of 12 to 19 residues is used as the polypeptide linker that links these variable regions.

In order to obtain scFv-encoding DNA, of the sequences of DNA encoding the heavy chain or heavy chain variable region of the antibody and DNA encoding the light chain or light chain variable region thereof, each DNA portion encoding the whole or desired amino acid sequence is used as a template and amplified by PCR using a primer pair flanking both ends of the template. Subsequently, DNA encoding the polypeptide linker moiety is further amplified in combination with a primer pair flanking both ends thereof to link them to the heavy and light chains, respectively.

The scFv-encoding DNA can be used to thereby prepare, according to a routine method, an expression vector containing the DNA and host cells transformed with the expression vector. In addition, the host cells are cultured, and the scFv can be collected from the cultures according to a routine method.

Also in order to obtain other functional fragments of the antibody, a gene encoding each functional fragment is obtained according to the method described above, and cells are transfected with the gene. The functional fragment of interest can be collected from cultures of the cells.

The antibody of the present invention may be multimerized to thereby enhance its affinity for the antigen. The antibodies to be multimerized may be antibodies of one type or may be a plurality of antibodies recognizing a plurality of epitopes, respectively, or the same antigen. Examples of antibody multimerization methods can include the binding of two scFvs to an IgG CH3 domain, the binding thereof to streptavidin, and the introduction of a helix-turn-helix motif.

The antibody of the present invention may be a mixture of plural types of anti-ROBO4 antibodies differing in amino acid sequence, i.e., a polyclonal antibody. Examples of the polyclonal antibody can include a mixture of plural types of antibodies differing in a portion or the whole of the CDR set. Such a polyclonal antibody can be collected from cultures of mixed-cultured different antibody-producing cells (WO2004/061104). Moreover, separately prepared antibodies may be mixed. Furthermore, antiserum, which is one aspect of the polyclonal antibody, can be prepared by immunizing animals with the desired antigen and collecting serum from the animals according to a standard method.

Antibodies conjugated with various molecules such as polyethylene glycol (PEG) can also be used as modified forms of the antibody.

The antibody of the present invention may further be any of conjugates formed by these antibodies with other drugs (immunoconjugates). Examples of such an

antibody can include the antibody conjugated with a radioactive material or a compound having a pharmacological effect (Nature Biotechnology (2005) 23, p. 1137-1146).

(4-7) Purification of antibody

The obtained antibody can be purified into a homogeneous level. Usual protein separation and purification methods can be used for the separation and purification of the antibody.

The antibody can be separated and purified by appropriately selected or combined approaches, for example, chromatography columns, filters, ultrafiltration, salting out, dialysis, preparative polyacrylamide gel electrophoresis, and/or isoelectric focusing (Strategies for Protein Purification and Charcterization: A Laboratoy Course Manual, Daniel R. Marshak et al. eds., Cold Spring Harbor Laboratory Press (1996); Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)) but not limited to them.

Examples of chromatography include affinity chromatography, ion-exchange chromatography, hydrophobic chromatography, gel filtration, reverse-phase chromatography, and adsorption chromatography.

These chromatography approaches can be performed using liquid-phase chromatography such as HPLC or FPLC.

Examples of columns used in affinity chromatography can include protein A, protein G, and antigen columns.

Examples of the protein A column include Hyper D (manufactured by Pall Corp.), POROS (manufactured by Applied Biosystems, Inc.), and Sepharose F.F. (manufactured by GE Healthcare Bio-Sciences Crop.).

Also, the antibody may be purified based on its binding activity against the antigen using an antigen-immobilized carrier.

The present invention provides even a gene encoding the antibody of the present invention or the functional fragment thereof, or the modified form thereof, a recombinant vector containing an insert of the gene, a cell transfected with the gene or the vector, and a cell producing the antibody of the present invention.

An antibody or functional fragment thereof which is produced by any of the methods (4-1) to (4-6) can be included in the present invention.

5. Pharmaceutical composition

The present invention provides a pharmaceutical composition comprising the anti-ROBO4 antibody or the functional fragment thereof, or the modified form thereof.

The pharmaceutical composition of the present invention is useful in the treatment or prevention of a disease that shows angiogenesis as one of pathological findings during the course of onset, progression, and/or exacerbation and can be improved by the suppression of this angiogenesis or vascular permeability (hereinafter, this disease is referred to as an "angiogenic disease" for the sake of convenience). Examples of the angiogenic disease can include exudative age-related macular degeneration, diabetic retinopathy, macular edema, benign or malignant tumor, atherosclerosis, retrolental fibroplasia, angioma, chronic inflammation, ocular neovascular disease, proliferative retinopathy, neovascular glaucoma, immune rejection of a corneal tissue transplant or other tissue transplants, rheumatoid arthritis, psoriasis, acute inflammation, sepsis, and obesity. The pharmaceutical composition of the present invention is useful as an agent in the treatment or prevention of an angiogenic disease, preferably useful in the treatment or prevention of exudative age-related macular degeneration, diabetic retinopathy, macular edema, benign or malignant tumor, retrolental fibroplasia, ocular neovascular disease, proliferative retinopathy, neovascular glaucoma, and immune rejection of a corneal tissue transplant, more preferably useful in the treatment or prevention of exudative age-related macular degeneration, diabetic

retinopathy, macular edema, benign or malignant tumor, retrolental fibroplasia, ocular neovascular disease, proliferative retinopathy and neovascular glaucoma.

In the present invention, the treatment and/or treatment of a disease includes, but not limited to, the prevention of onset of the disease, preferably the disease in an individual having the expressed ROBO4 protein, the suppression or inhibition of exacerbation or progression thereof, the alleviation of one or two or more symptoms exhibited by an individual affected with the disease, the suppression or remission of exacerbation or progression thereof, the treatment or prevention of a secondary disease, and the like.

The pharmaceutical composition of the present invention can contain a therapeutically or preventively effective amount of the anti-ROBO4 antibody or the functional fragment of the antibody and a pharmaceutically acceptable diluent, vehicle, solubilizer, emulsifier, preservative, and/or additive.

The "therapeutically or preventively effective amount" means an amount that exerts therapeutic or preventive effects on a particular disease by means of a particular dosage form and administration route.

The pharmaceutical composition of the present invention may contain materials for changing, maintaining, or retaining pH, osmotic pressure, viscosity, transparency, color, tonicity, sterility, or the stability, solubility, sustained release, absorbability, permeability, dosage form, strength, properties, shape, etc., of the composition or the antibody contained therein (hereinafter, referred to as "pharmaceutical materials"). The pharmaceutical materials are not particularly limited as long as they are pharmacologically acceptable materials. For example, no or low toxicity is a property preferably possessed by these pharmaceutical materials.

Examples of the pharmaceutical materials can include, but not limited to, the followings: amino acids such as glycine, alanine, glutamine, asparagine, histidine, arginine, and lysine; antimicrobial agents; antioxidants such as ascorbic acid, sodium

sulfate, and sodium bisulfite; buffers such as phosphate, citrate, or borate buffers, sodium bicarbonate, and Tris-HCl solutions; fillers such as mannitol and glycine; chelating agents such as ethylenediaminetetraacetic acid (EDTA); complexing agents such as caffeine, polyvinylpyrrolidine, β-cyclodextrin, and hydroxypropyl-β-cyclodextrin; bulking agents such as glucose, mannose, and dextrin; other hydrocarbons such as monosaccharides, disaccharides, glucose, mannose, and dextrin; coloring agents; corrigents; diluents; emulsifiers; hydrophilic polymers such as polyvinylpyrrolidine; low-molecular-weight polypeptides; salt-forming counterions; antiseptics such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, and hydrogen peroxide; solvents such as glycerin, propylene glycol, and polyethylene glycol; sugar alcohols such as mannitol and sorbitol; suspending agents; surfactants such as PEG, sorbitan ester, polysorbates such as polysorbate 20 and polysorbate 80, triton, tromethamine, lecithin, and cholesterol; stability enhancers such as sucrose and sorbitol; elasticity enhancers such as sodium chloride, potassium chloride, mannitol, and sorbitol; transport agents; diluents; excipients; and/or pharmaceutical additives.

The amount of these pharmaceutical materials added is 0.001 to 1000 times, preferably 0.01 to 100 times, more preferably 0.1 to 10 times the weight of the anti-ROBO4 antibody or the functional fragment thereof, or the modified form thereof.

A pharmaceutical composition containing an immunoliposome comprising the anti-ROBO4 antibody or the functional fragment thereof, or the modified form thereof encapsulated in a liposome or a modified antibody form comprising the antibody conjugated with a liposome (U.S. Patent No. 6214388, etc.) is also included in the pharmaceutical composition of the present invention.

The excipients or vehicles are not particularly limited as long as they are liquid or solid materials usually used in injectable water, saline, artificial cerebrospinal fluids,

and other preparations for oral or parenteral administration. Examples of the saline can include neutral saline and serum albumin-containing saline.

Examples of the buffers can include Tris buffers adjusted to bring about the final pH of the pharmaceutical composition to 7.0 to 8.5, acetate buffers adjusted to bring about the final pH thereof to 4.0 to 5.5, citrate buffers adjusted to bring about the final pH thereof to 5.0 to 8.0, and histidine buffers adjusted to bring about the final pH thereof to 5.0 to 8.0.

The pharmaceutical composition of the present invention is a solid, a liquid, a suspension, or the like. Another example of the pharmaceutical composition of the present invention can include freeze-dried preparations. The freeze-dried preparations can be formed using an excipient such as sucrose.

The administration route of the pharmaceutical composition of the present invention may be any of enteral administration, local administration, and parenteral administration and may be selected preferably according to the targeted disease. Specific examples thereof can include intravenous administration, intraarterial administration, intramuscular administration, intradermal administration, hypodermic administration, intraperitoneal administration, transdermal administration, intraosseous administration, and intraarticular administration. Also, intraocular administration can be used preferably for an ophthalmic angiogenic disease such as exudative age-related macular degeneration, diabetic retinopathy, macular edema, retrolental fibroplasia, ocular neovascular disease, proliferative retinopathy, neovascular glaucoma, or immune rejection of a corneal tissue transplant.

The recipe of the pharmaceutical composition can be determined according to the administration method, the binding affinity of the antibody for the ROBO4 protein, etc. The anti-ROBO4 antibody of the present invention or the functional fragment thereof, or the modified form thereof having higher affinity (lower K_D value) for the ROBO4 protein can exert its drug efficacy at a lower dose.

The dose of the anti-ROBO4 antibody of the present invention can be determined appropriately according to the species of an individual, the type of a disease, symptoms, sex, age, pre-existing conditions, the binding affinity of the antibody for the ROBO4 protein or its biological activity, and other factors. A dose of usually 0.01 to 1000 mg/kg, preferably 0.1 to 100 mg/kg, can be administered once every day to 180 days or twice or three or more times a day.

Examples of the form of the pharmaceutical composition can include injections (including freeze-dried preparations and drops), suppositories, transnasal absorption preparations, transdermal absorption preparations, sublingual formulations, capsules, tablets, ointments, granules, aerosols, pills, powders, suspensions, emulsions, eye drops, and biological implant formulations.

The pharmaceutical composition comprising the anti-ROBO4 antibody of the present invention or the functional fragment thereof, or the modified form thereof as an active ingredient may be used in combination with a further therapeutic or prophylactic agent. Examples of said agent include an anti-angiogenesis drug, anti-inflammatory drug, and/or an anticancer drug. For example, the anti-angiogenesis drug, anti-inflammatory drug, and/or anticancer drug is administered to a subject, and then, the pharmaceutical composition comprising the anti-ROBO4 antibody or the functional fragment of the antibody as an active ingredient is administered thereto. Alternatively, the pharmaceutical composition is administered to a subject, and then, the anti-angiogenesis drug, anti-inflammatory drug, and/or anticancer drug is administered thereto. Alternatively, the pharmaceutical composition may be administered to a subject simultaneously with the anti-angiogenesis drug, anti-inflammatory drug, and/or anticancer drug. Examples of the anti-angiogenesis drug can include ranibizumab.

The present invention provides even a method for treating or preventing an angiogenic disease such as exudative age-related macular degeneration, diabetic retinopathy, macular edema, benign or malignant tumor, atherosclerosis, retrolental

fibroplasia, angioma, chronic inflammation, ocular neovascular disease, proliferative retinopathy, neovascular glaucoma, immune rejection of a corneal tissue transplant or other tissue transplants, rheumatoid arthritis, psoriasis, acute inflammation, sepsis, or obesity, use of the antibody of the present invention for the preparation of a pharmaceutical composition for the treatment or prevention of the angiogenic disease, and use of the antibody of the present invention for the treatment or prevention of the angiogenic disease. A kit for treatment or prevention comprising the antibody of the present invention is also included in the present invention.

6. Composition for diagnosis

The present invention provides a composition for examination or diagnosis comprising the anti-ROBO4 antibody of the present invention or the functional fragment thereof, or the modified form thereof (hereinafter, collectively referred to as a "composition for diagnosis").

The composition for diagnosis of the present invention is useful in the examination or diagnosis of an angiogenic disease such as exudative age-related macular degeneration, diabetic retinopathy, macular edema, benign or malignant tumor, atherosclerosis, retrolental fibroplasia, angioma, chronic inflammation, ocular neovascular disease, proliferative retinopathy, neovascular glaucoma, immune rejection of a corneal tissue transplant or other tissue transplants, rheumatoid arthritis, psoriasis, acute inflammation, sepsis, or obesity. The composition for diagnosis of the present invention is also useful in the examination or diagnosis of early angiogenesis or pre-angiogenesis symptoms, which do not satisfy the conventional diagnosis criteria, undiagnosed symptoms that evolve to angiogenesis, etc. In the present invention, the examination or the diagnosis includes, for example, the determination or testing of a risk of acquiring a disease, the determination of the presence or absence of a disease, the testing of the degree of progression or exacerbation, the testing or determination of the

effect of drug therapy using the pharmaceutical composition comprising the anti-ROBO4 antibody or the like, the testing or determination of the effect of therapy other than drug therapy, the testing of a risk of recurrence, and the determination of the presence or absence of recurrence. However, the examination or the diagnosis according to the present invention is not limited to them as long as it is usual examination or diagnosis.

When the ROBO4 protein is detected in a 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20-fold or more amount, preferably 10-fold or more amount, in a sample derived from a test subject compared with a sample derived from a healthy individual, the test subject can be diagnosed as having an angiogenic disease or as being at a high risk of acquiring it. Moreover, when the serum concentration of the ROBO4 protein exceeds a particular reference value, the test subject is diagnosed as having an angiogenic disease or can be diagnosed as being at a high risk of acquiring it. The reference value is usually 0.01 to 10 ng/ml, preferably 0.1 to 1 ng/ml, more preferably 0.1 to 0.3 ng/ml.

Such a composition for diagnosis can contain a pH buffer, an osmoregulator, salts, a stabilizer, an antiseptic, a color developer, a sensitizer, an aggregation inhibitor, and the like.

The present invention provides even a method for examining or diagnosing an angiogenic disease such as exudative age-related macular degeneration, diabetic retinopathy, macular edema, benign or malignant tumor, atherosclerosis, retrolental fibroplasia, angioma, chronic inflammation, ocular neovascular disease, proliferative retinopathy, neovascular glaucoma, immune rejection of a corneal tissue transplant or other tissue transplants, rheumatoid arthritis, psoriasis, acute inflammation, sepsis, or obesity, use of the antibody of the present invention for the preparation of a composition for the diagnosis of the angiogenic disease, and use of the antibody of the present invention for the examination or diagnosis of the angiogenic disease. A kit for

examination or diagnosis comprising the antibody of the present invention is also included in the present invention.

The examination or diagnosis method involving the antibody of the present invention is preferably sandwich ELISA. A usual detection method using antibodies, such as ELISA, RIA, ELISPOT (enzyme-linked immunospot) assay, dot blotting, an Ouchterlony test, or CIE (counterimmunoelectrophoresis), may be used. Antibodies applied to the sandwich ELISA assay system may be any combination of two antibodies that recognize ROBO4, but do not compete with each other. In addition to biotin, a labeling method that can be carried out in biochemical analysis, such as HRP, alkaline phosphatase, or FITC, can be used as a labeling method for the antibodies. A chromogenic substrate such as TMB (3, 3', 5, 5'-tetramethylbenzidine), BCIP (5-bromo-4-chloro-3-indolyl phosphate), ρ-NPP (ρ-nitrophenyl phosphate), OPD (o-Phenylenediamine), ABTS (3-Ethylbenzothiazoline-6-sulfonic acid), SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Fisher Scientific Inc.), a fluorescent substrate such as QuantaBlu(TM) Fluorogenic Peroxidase Substrate (Thermo Fisher Scientific Inc.), and a chemiluminescent substrate can be used in detection using enzymatic labeling. Samples derived from human or non-human animals as well as artificially treated samples such as recombinant proteins can be subjected to this assay. Examples of test samples derived from organism individuals can include, but not limited to, blood, synovial fluids, ascites, lymph, cerebrospinal fluids, and tissue homogenate supernatants.

The sandwich ELISA kit for examination or diagnosis comprising the antibody of the present invention may contain a solution of ROBO4 protein standards, a coloring reagent, a buffer solution for dilution, an antibody for solid phase, antibody for detection, and a washing solution, and the like. The amount of the antibody bound to the antigen can be measured preferably using a method such as an absorbance, fluorescence, luminescence, or RI (radioisotope) method. An absorbance plate reader,

a fluorescence plate reader, a luminescence plate reader, an RI liquid scintillation counter, or the like is preferably used in the measurement.

[Examples]

Hereinafter, the present invention will be described in more detail with reference to Examples. However, the present invention is not intended to be limited to them.

In the Examples below, each operation for genetic engineering was performed by methods described in "Molecular Cloning" (Sambrook, J., Fritsch, E.F., and Maniatis, T., Cold Spring Harbor Laboratory Press, 1989) or methods described in other experimental manuals used by those skilled in the art, or performed according to the instructions of commercially available reagents or kits used, unless otherwise specified.

(Example 1) Preparation of expression vector

1)-1 Preparation of human ROBO4 expression vector

1)-1-1 Preparation of full-length human ROBO4 expression vector

Human ROBO4 cDNA was cleaved off with EcoRV and NotI from a plasmid (manufactured by Open Biosystems) comprising human ROBO4 cDNA (Accession No. BC039602) and incorporated between EcoRV and NotI of a pCI vector (manufactured by Promega Corp.) to prepare a full-length human ROBO4 expression vector (hereinafter, referred to as "pCI-hROBO4"). The sequence of the human ROBO4 gene cloned in this vector is shown in SEQ ID NO: 1 . Also, the amino acid sequence of human ROBO4 is shown in SEQ ID NO: 2 .

1)-1-2 Preparation of human ROBO4 extracellular region expression vector

cDNA encoding a human ROBO4 extracellular region polypeptide (consisting of an amino acid sequence represented by amino acid Nos. 1 to 461 of SEQ ID NO: 2 ;

hereinafter, abbreviated to "human ROBO4-ECD") was amplified through PCR reaction using a primer set:

primer 1F: 5'-aaaggtaccaccatgggctctggaggagacagcctcctg-3' and

primer 1R: 5'-aaagatatcctgctccagggtccagggaccatgctcact-3'

The obtained PCR product was cloned into a pEF6/V5-His-TOPO vector (manufactured by Life Technologies Corp.) (hereinafter, the resulting vector is abbreviated to "pEF6-ROBO4-ECD"; hereinafter, a recombinant protein expressed by "pEF6-ROBO4-ECD" is referred to as "rROBO4-ECD").

1)-1-3 Preparation of N-terminal FLAG-tagged full-length human ROBO4 and human ROBO4 extracellular region/domain deletion variant expression vectors

In order to construct vectors for expression of a protein comprising a region consisting of an amino acid sequence represented by amino acid Nos. 28 to 1007 of SEQ ID NO: 2 of human ROBO4 (in the diagram, this region is referred to as "hROBO4-28"), a region consisting of an amino acid sequence represented by amino acid Nos. 46 to 1007 thereof (in the diagram, this region is referred to as "hROBO4-46"), a region consisting of an amino acid sequence represented by amino acid Nos. 132 to 1007 thereof (in the diagram, this region is referred to as "hROBO4-132"), a region consisting of an amino acid sequence represented by amino acid Nos. 210 to 1007 thereof (in the diagram, this region is referred to as "hROBO4-210"), a region consisting of an amino acid sequence represented by amino acid Nos. 225 to 1007 thereof (in the diagram, this region is referred to as "hROBO4-225"), or a region consisting of an amino acid sequence represented by amino acid Nos. 341 to 1007 thereof (in the diagram, this region is referred to as "hROBO4-341") with the N-terminus tagged with FLAG, PCR reaction was performed with pCI-hROBO4 as a template using each primer set:

primer set for hROBO4-28 amplification:

primer 2F: 5'-

ggtaccgccatgggctctggaggagacagcctcctcggcggcagaggttccctgcctctgctgctcctgctcatcatgggag

gcatggctgattacaaggatgacgacgataagcaggactccccgccccagatcctagtccac-3' and

primer 2R: 5'-gctagcggagtaatctacaggagaagcaccagccttg-3',

primer set for hROBO4-46 amplification:

primer 3F: 5'-

ggtaccgccatgggctctggaggagacagcctcctcggcggcagaggttccctgcctctgctgctcctgctcatcatgggag

gcatggctgattacaaggatgacgacgataagcctggccctgccaggatgagctgccaag-3' and the primer 2R

primer set for hROBO4-132 amplification:

primer 4F: 5'-

ggtaccgccatgggctctggaggagacagcctcctcggcggcagaggttccctgcctctgctgctcctgctcatcatgggag

gcatggctgattacaaggatgacgacgataaggtggctgtcctccgggaggatttccagatc-3' and the primer 2R,

primer set for hROBO4-210 amplification:

primer 5F: 5'-

ggtaccgccatgggctctggaggagacagcctcctcggcggcagaggttccctgcctctgctgctcctgctcatcatgggag

gcatggctgattacaaggatgacgacgataagaccaacagcgcaggacatagggagagcc-3' and the primer 2R,

primer set for hROBO4-225 amplification:

primer 6F: 5'-

ggtaccgccatgggctctggaggagacagcctcctcggcggcagaggttccctgcctctgctgctcctgctcatcatgggag

gcatggctgattacaaggatgacgacgataagatccaggagccccaggactacacggagcc-3' and the primer 2R,

primer set for hROBO4-341 amplification:

primer 7F: 5'-

ggtaccgccatgggctctggaggagacagcctcctcggcggcagaggttccctgcctctgctgctcctgctcatcatgggag

gcatggctgattacaaggatgacgacgataagaggctgccggaaaaagtgcccagtgcccca-3' and the primer

2R. The obtained PCR product was incorporated into a pCR4Blunt-TOPO vector (manufactured by Life Technologies Corp.) to prepare a cloning vector. From each cloning vector, the corresponding cDNA was cleaved off with KpnI and NheI and

incorporated between KpnI and NheI of a pCI vector to prepare an N-terminal FLAG-tagged full-length human ROBO4 expression vector and four human ROBO4 extracellular region/domain deletion variant expression vectors. The N-terminal FLAG-tagged full-length human ROBO4 vector consists of a nucleotide encoding the signal sequence of ROBO4 (amino acids of amino acid Nos. 1 to 27 of SEQ ID NO: 2) + FLAG sequence (DYKDDDDK) + ROBO4 (amino acids of amino acid Nos. 28 to 1007 of SEQ ID NO: 2) from the N-terminus. Hereinafter, the vector for expression of N-terminal FLAG-tagged full-length human ROBO4 is referred to as "pCI-FLAG-hROBO4-28". Of the human ROBO4 extracellular region/domain deletion variant expression vectors, for example, the vector for expression of ROBO4 consisting of amino acid Nos. 46 to 1007 of SEQ ID NO: 2 consists of a nucleotide encoding the signal sequence of ROBO4 (amino acids of amino acid Nos. 1 to 27 of SEQ ID NO: 2) + FLAG sequence (DYKDDDDK) + extracellular region-deleted ROBO4 (amino acids of amino acid Nos. 46 to 1007 of SEQ ID NO: 2). This vector is referred to as "pCI-hROBO4-46". Likewise, the vectors encoding ROBO4 having a partial deletion in the extracellular region of ROBO4 are referred to as "pCI-hROBO4-132", "pCI-hROBO4-210", "pCI-hROBO4-225", and "pCI-hROBO4-341", respectively.

The nucleotide sequence encoding the amino acid sequence of FLAG-tagged full-length human ROBO4 or each extracellular region/domain deletion variant of human ROBO4 cloned in the vector is shown in SEQ ID NO: 3, 5, 7, 9, 11, or 13 . Also, the amino acid sequence of the corresponding FLAG-tagged full-length human ROBO4 or extracellular region/domain deletion variant of human ROBO4 is shown in SEQ ID NO: 4, 6, 8, 10, 12, or 14 .

1)-1-4 Preparation of human ROBO4 intracellular region deletion variant expression vector

In order to construct a vector for expression of a protein comprising a region consisting of an amino acid sequence represented by amino acid Nos. 1 to 511 of SEQ ID NO: 2 of human ROBO4 (hereinafter, this region is referred to as "hROBO4-ΔC"), a stop codon was inserted immediately after a codon encoding the 511th amino acid of human ROBO4 with pCI-hROBO4 as a template using primer set for hROBO4-ΔC:
primer 8F: 5'-cagatataccagtgaggatgcctgaatcctaaaacacaggatggatc-3' and
primer 8R: 5'-gatccatcctgtgttttaggattcaggcatcctcactggtatatctg-3',
and QuikChange XL Site-Directed Mutagenesis Kit (manufactured by Agilent Technologies, Inc.) to prepare a hROBO4-ΔC expression vector (hereinafter, referred to as "pCI-hROBO4-ΔC").

1)-2 Preparation of mouse ROBO4 expression vector

PCR reaction was performed with Mouse Heart QUICK-Clone cDNA (manufactured by Takara Bio Inc.) as a template using a primer set:
primer 9F: 5'-ggtaccgccatgggacaaggagaggagccgagagcagccatg-3' and
primer 9R: 5'-gcggccgcggaggaatcaccagccttgggcacagcaccag-3'
The obtained PCR product was incorporated into a pCR-Blunt II-TOPO vector (manufactured by Life Technologies Corp.) to prepare a cloning vector comprising mouse ROBO4 cDNA. From the cloning vector, mouse ROBO4 cDNA was cleaved off with KpnI and NotI and incorporated between KpnI and NotI of a pCI vector to prepare a mouse ROBO4 expression vector (hereinafter, referred to as "pCI-mROBO4"). The sequence of an ORF site in the mouse ROBO4 gene cloned in this vector is shown in nucleotide Nos. 7 to 3051 of SEQ ID NO: 15 . Also, the amino acid sequence of mouse ROBO4 is shown in SEQ ID NO: 16 .

1)-3 Preparation of rat ROBO4 expression vector

PCR reaction was performed with Rat Spleen QUICK-Clone cDNA (manufactured by Takara Bio Inc.) as a template using a primer set:
primer 10F: 5'-ggtaccgccatgggacaaggagaggagctgagagcagcc-3' and
primer 10R: 5'-gcggccgcggaggaatcaccagccttgggcacaacacc-3'
The obtained PCR product was incorporated into a pCR4Blunt-TOPO vector to prepare a cloning vector comprising rat ROBO4 cDNA. From the cloning vector, rat ROBO4 cDNA was cleaved off with KpnI and NotI and incorporated between KpnI and NotI of a pCI vector to prepare a rat ROBO4 expression vector (hereinafter, referred to as "pCI-raROBO4"). The nucleotide sequence of rat ROBO4 cDNA is shown in SEQ ID NO: 17 . The amino acid sequence encoded by this nucleotide sequence is shown in SEQ ID NO: 18 .

1)-4 Preparation of N-terminal FLAG-tagged cynomolgus monkey ROBO4 expression vector

PCR reaction was performed with cDNA synthesized from cynomolgus monkey kidney total RNA as a template using a primer set:
primer 11F: 5'-ggtaccgccatgggctctggaggagaaagcctccggg-3' and
primer 11R: 5'-ggagtaatctacaggagaagcaccagccttg-3'
The obtained PCR product was incorporated into a pCR4Blunt-TOPO vector to prepare two types of cloning vectors comprising each cynomolgus monkey ROBO4 cDNA (hereinafter, referred to as cynoROBO4-1 or cynoROBO4-2) (hereinafter, these vectors are referred to as pCR-cynoROBO4-1 and pCR-cynoROBO4-2, respectively).

Next, PCR reaction was performed with pCR-cynoROBO4-1 or pCR-cynoROBO4-2 as a template using a primer set:
primer 12F: 5'-ggatccgccatgggctctggaggagaaagcctccg-3' and
primer 12R: 5'-gcggccgctcaggagtaatctacaggagaagcaccagccttg-3'

The obtained PCR product was incorporated intoa pCR4Blunt-TOPO vector to prepare a cloning vector comprising each cynomolgus monkey ROBO4 cDNA. From the cloning vector, the corresponding cynomolgus monkey ROBO4 cDNA was cleaved off with BamHI and NotI and incorporated between BamHI and NotI of a pCI vector to prepare two types of cynomolgus monkey ROBO4 expression vectors (hereinafter, referred to as pCI-cynoROBO4-1 and pCI-cynoROBO4-2, respectively).

Next, PCR reaction was performed with pCI-cynoROBO4-1 or pCI-cynoROBO4-2 as a template using a primer set:

primer 13F: 5'-

ggtaccgccatgggctctggaggagaaagcctccgaggctcccgggcttcccggcctctgctgctcctgctcatcatgggag gcatggctgattacaaggatgacgacgataagcaggactccccgccccagatcctagtccac-3'

and the primer 12R.

The obtained PCR product was incorporated into a pCR-TOPO vector (manufactured by Life Technologies Corp.) to prepare each cloning vector comprising N-terminal FLAG-tagged cynomolgus monkey ROBO4 cDNA. From the cloning vector, the corresponding cynomolgus monkey ROBO4 cDNA was cleaved off with KpnI and NotI and incorporated between KpnI and NotI of a pCI vector to prepare N-terminal FLAG-tagged cynomolgus monkey ROBO4 expression vectors (hereinafter, referred to as "pCI-FLAG-cynoROBO4-1" and "pCI-FLAG-cynoROBO4-2", respectively). The nucleotide sequence of cynomolgus monkey ROBO4 cDNA cloned in each of pCI-FLAG-cynoROBO4-1 and pCI-FLAG-cynoROBO4-2 is shown in SEQ ID NOs: 19 and 21, respectively, . The amino acid sequence encoded by each nucleotide sequence is shown in SEQ ID NOs: 20 and 22, respectively, .

1)-5 Preparation of N-terminal FLAG-tagged human ROBO1 expression vector

PCR reaction was performed with Human Heart QUICK-Clone cDNA (manufactured by Takara Bio Inc.) as a template using a primer set:

primer 13F: 5'-ggggacaagtttgtacaaaaaagcaggcttcaccatgattgcggagcccgctcacttttacctg-3'
and
primer 13R: 5'-ggggaccactttgtacaagaaagctgggtcgctttcagtttcctctaattcttc-3'
The obtained PCR product and a pDONR221 vector (manufactured by Life Technologies Corp.) were subjected to BP reaction to prepare a donor vector comprising human ROBO1 cDNA.

Next, the PCR reaction was performed with the donor vector as a template using a primer set:
primer 14F: 5'-
gcggccgcatgattgcggagcccgctcacttttacctgtttggattaatatgtctctgttcaggctcccgtcttgattacaaggatg
acgacgataagcgtcaggaagattttccacctcgcattgttg-3' and
primer 14R: 5'-gctagctcagctttcagtttcctctaattcttc-3'
The obtained PCR product was incorporated intoa pCR4Blunt-TOPO vector to prepare a cloning vector comprising N-terminal FLAG-tagged human ROBO1 cDNA. From the cloning vector, N-terminal FLAG-tagged human ROBO1 cDNA was cleaved off with NheI and NotI and incorporated between NheI and NotI of a pCI vector to prepare an expression vector (hereinafter, referred to as "pCI-FLAG-hROBO1"). The nucleotide sequence of N-terminal FLAG-tagged human ROBO1 cDNA is shown in SEQ ID NO: 23 . The amino acid sequence encoded by this nucleotide sequence is shown in SEQ ID NO: 24 .

1)-6 Preparation of human ROBO2 expression vector

PCR reaction was performed with Human Lung QUICK-Clone cDNA (manufactured by Takara Bio Inc.) as a template using a primer set:
primer 15F: 5'-gcggccgcatgagtctgctgatgtttacacaactactg-3' and
primer 15R: 5'-gctagcctataattcacctgtaaactgtccttgactgttg-3'

The obtained PCR product was incorporated to a pCR4Blunt-TOPO vector to prepare a cloning vector comprising human ROBO2 cDNA. From the cloning vector, human ROBO2 cDNA was cleaved off with NotI and NheI and incorporated between NotI and NheI of a pCI vector to prepare an expression vector (hereinafter, referred to as "pCI-hROBO2"). The nucleotide sequence of human ROBO2 cDNA is shown in SEQ ID NO: 25 . The amino acid sequence encoded by this nucleotide sequence is shown in SEQ ID NO: 26 .

1)-7 Preparation of human ROBO3 expression vector

PCR reaction was performed with human ROBO3/pENTR223.1 (manufactured by Open Biosystems) as a template using a primer set:
primer 16F: 5'-gcggccgcatgctgcgctacctgctgaaaacgctgctg-3' and
primer 16R: 5'-gctagctcatcttggttcctctcggcgtttctgtcc-3'
The obtained PCR product was incorporated to a pCR4Blunt-TOPO vector to prepare a cloning vector comprising human ROBO3 cDNA. From the cloning vector, human ROBO3 cDNA was cleaved off with NotI and NheI and incorporated between NotI and NheI of a pCI vector to prepare an expression vector (hereinafter, referred to as "pCI-hROBO3"). The sequence of an ORF site in the human ROBO3 gene cloned in this vector is shown in nucleotide Nos. 35 to 4192 of SEQ ID NO: 27 . Also, the amino acid sequence of human ROBO3 is shown in SEQ ID NO: 28 .

(Example 2) Preparation of monoclonal antibody

2)-1 Preparation of antigenic protein

In order to express rROBO4-ECD, FreeStyle 293-F cells (manufactured by Life Technologies Corp.) were transfected with pEF6-ROBO4-ECD using 293fectin (manufactured by Life Technologies Corp.) and cultured at 37°C for 6 days under 8% CO_2 conditions. After completion of the culture, the culture solution was collected by

centrifugation and used as a rROBO4-ECD purification stock. The obtained culture supernatant was dialyzed against 20 mM Tris-HCl, pH 7.5 using a dialysis tube having a molecular weight cutoff of 15000, filtered through a filter (0.45 μm), and then added to HiTrap 16/10 Q XL (manufactured by GE Healthcare Bio-Sciences Crop.) equilibrated with 20 mM Tris-HCl, pH 7.5. Elution was performed with a NaCl gradient (20 mM Tris-HCl, pH 7.5/0.2 M NaCl, 20 mM Tris-HCl, pH 7.5/1 M NaCl). A portion of the elution fraction was separated by SDS-polyacrylamide gel electrophoresis (hereinafter, abbreviated to "SDS-PAGE"). Then, the gel was subjected to Coomassie Brilliant Blue staining (hereinafter, abbreviated to "CBB staining") and detection by Western blotting to confirm a fraction containing rROBO4-ECD. Next, the fraction containing rROBO4-ECD was collected and added to HiLoad 16/60 Superdex 75 pg (manufactured by GE Healthcare Bio-Sciences Crop.) equilibrated with PBS. After elution with PBS, a portion of the elution fraction was separated by SDS-PAGE. Then, the gel was subjected to CBB staining and detection by Western blotting to confirm a fraction containing rROBO4-ECD. The fraction containing rROBO4-ECD was collected and used as an antigen for immunization and as an antigen for binding affinity assay. The protein concentration was measured using BCA Protein Assay Reagent (manufactured by Pierce Biotechnology, Inc.).

2)-2 Immunization

Six-week-old female BALB/c mice were used. At day 0, 50 μg of a mixture of rROBO4-ECD and a Freund's complete adjuvant was hypodermically or intradermally administered to each mouse. At days 7, 14, and 21, 50 μg of a mixture of rROBO4-ECD and a Freund's incomplete adjuvant was hypodermically or intradermally administered to the mouse. At day 38, 50 μg of rROBO4-ECD was intraperitoneally administered to the mouse. At day 42, the mouse lymph node or spleen was collected and used in hybridoma preparation.

2)-3 Hybridoma preparation

The lymph node cells or spleen cells and mouse myeloma SP2/0-ag14 cells were electrically fused using Hybrimune Hybridoma Production System (manufactured by Cyto Pulse Sciences, Inc.), diluted with ClonaCell-HY Selection Medium D (manufactured by StemCell Technologies Inc.), and cultured. Hybridoma colonies that appeared were collected to prepare monoclonal hybridomas. Each hybridoma colony collected was cultured, and the obtained hybridoma culture supernatant was screened for anti-ROBO4 antibody-producing hybridomas.

2)-4 Antibody screening

2)-4-1 Preparation of antigen gene-expressing cell for Cell-ELISA

HEK293 cells were adjusted to 7.5×10^5 cells/mL in a DMEM medium containing 10% FBS. The cells were transfected with pCI-hROBO4 or a negative control pCI-mock using Lipofectamine 2000 (manufactured by Life Technologies Corp.), dispensed at a concentration of 50 μL/well to a 96-well half area plate (manufactured by Corning Inc.), and cultured overnight in a DMEM medium containing 10% FBS at 37°C under 5% CO_2 conditions. The obtained transfected cells were used in Cell-ELISA with them adhering to each other.

2)-4-2 Preparation of antigen gene-expressing cell for flow cytometry analysis

HEK293T cells were inoculated at a concentration of 1.125×10^7 cells/flask to a 225-cm^2 flask (manufactured by Sumitomo Bakelite Co., Ltd.) and cultured overnight in a DMEM medium containing 10% FBS at 37°C under 5% CO_2 conditions. On the next day, the HEK293T cells were transfected with pCI-ROBO4 or a negative control pCI-mock using Lipofectamine 2000 and further cultured overnight at 37°C under 5% CO_2 conditions. On the next day, the expression vector-transfected HEK293T cells

were treated with TrypLE Express (manufactured by Life Technologies Corp.), washed with DMEM containing 10% FBS, and then suspended in PBS containing 5% FBS. The obtained cell suspension was used in flow cytometry analysis.

2)-5 Cell-ELISA

After removal of a supernatant from the expression vector-transfected HEK293 cells prepared in 2)-4-1, the hybridoma culture supernatant was added to each of the pCI-hROBO4- and pCI-mock-transfected HEK293 cells, and the cells were left standing at 4°C for 1 hour. The cells in each well were washed once with PBS containing 5% FBS. Then, Anti-Mouse IgG-Peroxidase antibody produced in goat (manufactured by Sigma-Aldrich Corp.) diluted 500-fold with PBS containing 5% FBS was added thereto, and the cells were left standing at 4°C for 1 hour. The cells in each well were washed 5 times with PBS containing 5% FBS. Then, an OPD coloring solution (o-phenylenediamine dihydrochloride (manufactured by Wako Pure Chemical Industries, Ltd.) and H_2O_2 dissolved at concentrations of 0.4 mg/mL and 0.6% (v/v), respectively, in an OPD dissolving solution (0.05 M trisodium citrate, 0.1 M disodium hydrogen phosphate dodecahydrate, pH 4.5)) was added thereto at a concentration of 25 μL/well. Color reaction was performed with intermittent stirring and stopped by addition of 1 M HCl at a concentration of 25 μL/well. Then, absorbance at 490 nm was measured using a plate reader (ENVISION; manufactured by Perkin Elmer, Inc.). In order to select hybridomas producing an antibody specifically binding to ROBO4 expressed on cell membrane surface, hybridomas in the culture supernatants exhibiting higher absorbance in the pCI-hROBO4-transfected HEK293 cells compared with the negative control pCI-mock-transfected HEK293 cells were selected as anti-ROBO4 antibody production-positive hybridomas.

2)-6 Flow cytometry analysis

The antibody produced by each hybridoma determined to be positive in 2)-5 Cell-ELISA was further confirmed to bind to ROBO4 by flow cytometry. The HEK293T cell suspension prepared in 2)-4-2 was centrifuged. After removal of the supernatant, the hybridoma culture supernatant was added to each of the pCI-hROBO4-transfected cells and the pCI-mock-transfected cells, and the resulting suspension was left standing at 4°C for 1 hour. The cells were washed twice with PBS containing 5% FBS. Then, Anti-Mouse IgG FITC conjugate (manufactured by Sigma-Aldrich Corp.) diluted 1000-fold with PBS containing 5% FBS or Anti-Rat IgG FITC conjugate (manufactured by Sigma-Aldrich Corp.) diluted 320-fold with PBS containing 5% FBS was added thereto, and the resulting suspension was left standing at 4°C for 1 hour. The cells were washed three times with PBS containing 5% FBS, then resuspended in PBS containing 5% FBS and 2 μg/mL 7-aminoactinomycin D (manufactured by Molecular Probes), and subjected to detection using a flow cytometer (FC500; manufactured by Beckman Coulter, Inc.). Data was analyzed using Flowjo (manufactured by TreeStar Inc.). 7-aminoactinomycin D-positive dead cells were excluded by gating, and the histogram of FITC fluorescence intensity was then prepared for live cells. Hybridomas producing a sample in which the histogram of the pCI-ROBO4-transfected HEK293T cells was shifted to a stronger fluorescence intensity region compared with the histogram of the negative control pCI-mock-transfected 293T cells were obtained as anti-ROBO4 antibody-producing hybridomas. Anti-ROBO4 antibodies produced by the obtained hybridomas were designated as MAb1, MAb2, MAb3, and MAb4, respectively.

2)-7 Isotyping of monoclonal antibody

The isotype of each monoclonal antibody was determined using Mouse monoclonal isotyping kit or Rat monoclonal isotyping kit (manufactured by AbD Serotec). The results were IgG1 (MAb1 and MAb2) and IgG2b (MAb3 and MAb4).

2)-8 Preparation of monoclonal antibody

Each monoclonal antibody was purified from a hybridoma culture supernatant (hereinafter, referred to as an "antibody purification stock").

The antibody purification stock was prepared as follows: 8 to 9×10^7 hybridomas were inoculated to a 1272-cm^2 flask (manufactured by Corning Inc.) and cultured in a hybridoma SFM medium (manufactured by Life Technologies Corp.) containing 20% Ultra-LoW IgG fetal bovine serum at 37°C for 4 days under 5% CO_2 conditions, and the supernatant was then collected.

The antibody was purified using Hitrap Protein G HP or Hitrap MabSelect SuRe (manufactured by GE Healthcare Bio-Sciences Crop.). For Hitrap Protein G HP, the antibody purification stock was added to a column and washed with a binding buffer (0.02 M sodium phosphate, pH 7.0), followed by elution with 0.1 M glycine, pH 2.7. By contrast, for Hitrap MabSelect SuRe, the antibody purification stock was added to a column and washed with PBS, followed by elution with 2 M arginine-HCl, pH 4.0. The eluted antibody solution was neutralized, and the buffer was then replaced by PBS. The concentration of the antibody purified with Hitrap Protein G HP was measured using BCA Protein Assay Reagent. Mouse IgG2a (manufactured by R&D systems, Inc.) was used as a standard for a calibration curve. Alternatively, the concentration of the antibody purified with Hitrap MabSelect SuRe was determined by the measurement of absorbance (O.D. 280 nm) in an eluate of the antibody bound to POROS G 20 μm Column PEEK, 4.6 mm × 50 mm, 0.83 mL (manufactured by Applied Biosystems, Inc.). Specifically, the antibody sample diluted with PBS was added to POROS G 20 μm equilibrated with an equilibration buffer (30.6 mM sodium dihydrogen phosphate dodecahydrate, 19.5 mM monopotassium phosphate, 0.15 M NaCl, pH 7.0). The column was washed with an equilibration buffer, and an antibody bound to the column was then eluted with an eluent (0.1% (v/v) HCl, 0.15 M NaCl). The peak area of

absorbance (O.D.280 nm) in the eluate was measured, and the concentration was calculated according to the following equation: Antibody sample concentration (mg/mL) = (Peak area of antibody sample) / (Peak area of standard (human IgG1)) × Concentration of standard (mg/mL) × Dilution ratio of sample.

(Example 3) Detection of activation of ROBO4 downstream signal

3)-1 Preparation of reporter vector comprising interleukin-8 (IL-8) promoter region as response element

PCR reaction was performed with IL-8 promoter region DNA as a template using a primer set:

primer 17F: 5'-ggtaccgataaggaacaaataggaag-3' and

primer 17R: 5'-gagctcagcttgtgtgctctgctgtc-3'

The obtained PCR product was incorporated to a pCR4Blunt-TOPO vector to prepare a cloning vector comprising IL-8 promoter region (-253 to -59) DNA. From the cloning vector, IL-8 promoter region (-253 to -59) DNA was cleaved off with KpnI and SacI and incorporated between KpnI and SacI of a pGL4.15 vector (manufactured by Promega Corp.) to prepare a reporter vector comprising the IL-8 promoter region as a response element. The nucleotide sequence of IL-8 promoter region (-253 to -59) DNA is shown in SEQ ID NO: 29 .

3)-2 Reporter vector comprising Nuclear Factor-κB (NF-κB), Interferon gamma activation sequence (GAS), Interferon stimulated response element (ISRE), Transfection grade T cell factor (TCF)
as response element

pGL4.32[luc2P/NF-κB-RE/Hygro] Vector (manufactured by Promega Corp.), pGAS-TA-Luc Vector (manufactured by Takara Bio Inc.), pISRE-TA-Luc Vector (manufactured by Takara Bio Inc.), and TOPflash (manufactured by Millipore Corp.)

were respectively used as reporter vectors comprising NF-κB, GAS, ISRE, or TCF as a response element. Alternatively, response sequence-free pTA-Luc Vector (manufactured by Takara Bio Inc.) was used as a negative control. pRL-TK Vector (manufactured by Takara Bio Inc.) was used as an internal control.

3)-3 Analysis of signal varying in cell transiently expressing human ROBO4

HEK293 cells were inoculated at a concentration of 2×10^4 cells/well to a 96-well plate (coated with collagen I; manufactured by Asahi Glass Co., Ltd.) and cultured overnight in a DMEM medium containing 10% FBS at 37°C under 5% CO_2 conditions. On the next day, the HEK293 cells were transfected with pCI-ROBO4, pCI-ROBO4-ΔC, or a negative control pCI-mock and each of the reporter vectors shown in 3)-1 and 3)-2 using FuGene6 Transfection Reagent, and further cultured overnight at 37°C under 5% CO_2 conditions. On the next day, the firefly luciferase and Renilla luciferase activities of each well were determined as luminescence intensity in a plate reader (Mithras; manufactured by Berthold Technologies GmbH & Co, KG) using Dual-Glo Luciferase Assay System (manufactured by Promega Corp.), and the reporter activity of each well was calculated according to the following equation: Reporter activity = Firefly luciferase activity-derived luminescence intensity / Renilla luciferase activity-derived luminescence intensity. As a result, only the IL-8 promoter activity was increased in the pCI-hROBO4-transfected cells compared with the negative control pCI-mock-transfected cells (Figure 1). Moreover, this increase in the IL-8 promoter activity detected in the pCI-hROBO4-transfected cells was drastically attenuated in the cells transfected with pCI-hROBO4-ΔC (intracellular region deletion mutant of hROBO4), demonstrating that the increase in the IL-8 promoter activity detected in the pCI-hROBO4-transfected cells requires the intracellular region of ROBO4 (Figure 2). Accordingly, the increase in the IL-8 promoter activity in the pCI-hROBO4-transfected cells demonstrated that the activation of the ROBO4 downstream signal was detected.

(Example 4) Properties of MAb1

4)-1 Activation of ROBO4 downstream signal by MAb1

The pCI-hROBO4-transfected cells or the pCI-mock-transfected cells prepared in 3)-3 were cultured overnight. On the next day, each anti-ROBO4 antibody (MAb1, MAb2, MAb3, or MAb4) or a negative control Mouse IgG1 (manufactured by R&D systems, Inc.) was added thereto at concentrations of 0, 0.3125, 1.25, 5, and 20 μg/mL or 0, 0.25, 1, 4, and 16 μg/mL, and the cells were cultured at 37°C for 5 hours under 5% CO_2 conditions. Then, the firefly luciferase and Renilla luciferase activities of each well were determined as luminescence intensity in a plate reader (Mithras) using Dual-Glo Luciferase Assay System (manufactured by Promega Corp.), and the reporter activity of each well was calculated according to the following equation: Reporter activity = Firefly luciferase activity-derived luminescence intensity / Renilla luciferase activity-derived luminescence intensity. As a result, the negative control mouse IgG did not influence the IL-8 promoter activity in the cells transiently expressing human ROBO4, whereas MAb1 increased the IL-8 promoter activity (Figure 3). As in MAb1, MAb2 also increased the IL-8 promoter activity, whereas MAb3 or MAb4 did not increase the IL-8 promoter activity (Figure 4). In the pCI-mock cells, MAb1 or MAb2 did not increase the IL-8 promoter activity. These results demonstrated that MAb1 activated the downstream signal of ROBO4 and not all antibodies against ROBO4 activated the downstream signal of ROBO4.

MAb3 and MAb4 that were confirmed not to increase the activity of the ROBO4 downstream signal were evaluated for promoter activity in the presence of cross-linking antibodies (AffiPure Goat Anti-Mouse IgG Fc Fragment Specific, Cat NO. 115-005-071, Jackson ImmunoResearch) (two cross-linking antibody molecules with respect to one molecule of MAb3 or MAb4). As a result, increase in the promoter activity was observed for both the antibodies.

4)-2 HUVEC migration test

HUVEC (manufactured by KURABO INDUSTRIES LTD.) was cultured overnight in HuMedia-EB2 (manufactured by KURABO INDUSTRIES LTD.) containing 0.1% BSA at 37°C under 5% CO_2 conditions and then adjusted to 4×10^5 cells/mL with HuMedia-EB2 containing 0.1% BSA. 0.25 mL of the cell suspension having a concentration of 4×10^5 cells/mL was added to the upper layer of a chamber in BD Falcon FluoroBlok 24 multi-well insert system (Pore size: 8 μm) having a gelatin-coated membrane. Then, HuMedia-EB2 containing 0.1% BSA and 10 ng/mL human VEGF165 (manufactured by PeproTech Inc.) or human bFGF (BD Biosciences) and 2 μg/mL Mouse IgG2a or each anti-ROBO4 antibody (MAb1, MAb2, MAb3, or MAb4) was added to the lower layer of the chamber. After incubation at 37°C for 2 to 3 hours under 5% CO_2 conditions, HUVEC that migrated to the lower layer was stained with HuMedia-EB2 containing 4 μg/mL Calcein-AM (manufactured by Life Technologies Corp.) for 15 minutes. Then, the fluorescence intensity (excitation wavelength/fluorescence wavelength: 485 nm/538 nm) of each well was measured using a plate reader (FlexStation; Molecular Devices, LLC.), and the amount of migrating cells in each well was calculated according to the following equation: Amount of migrating cells = Fluorescence intensity of HUVEC-supplemented well - Fluorescence intensity of HUVEC-unsupplemented well. As a result, MAb1 suppressed the migration of HUVEC induced by VEGF or bFGF (Figure 5). As in MAb1, MAb2 also suppressed the migration of HUVEC induced by bFGF, whereas MAb3 or MAb4 did not suppress the cell migration (Figure 6). These results demonstrated that the increase in the IL-8 promoter activity by the anti-ROBO4 antibody correlated with the suppressive activity against HUVEC migration.

4)-3 Cross-species reactivity

4)-3-1 Preparation of antigen gene-expressing cell

HEK293 cells were inoculated at a concentration of 1.5×10^6 cells/dish to a 60-mm dish (manufactured by Corning Inc.) and cultured overnight in a DMEM medium containing 10% FBS at 37°C under 5% CO_2 conditions. On the next day, the HEK293 cells were transfected with pCI-hROBO4, pCI-mROBO4, pCI-raROBO4, pCI-FLAG-cynoROBO4-1, pCI-FLAG-cynoROBO4-2, pCI-hROBO1, pCI-hROBO2, or pCI-hROBO3 using FuGENE6 Transfection Reagent and further cultured overnight at 37°C under 5% CO_2 conditions. On the next day, the expression vector-transfected cells were treated with TrypLE Express (manufactured by Life Technologies Corp.), washed with PBS containing 5% FBS, and then suspended in PBS containing 5% FBS. The obtained cell suspension was used in flow cytometry analysis.

4)-3-2 Flow cytometry analysis

Each cell suspension prepared in 4)-3-1 was centrifuged, and the supernatant was removed. Then, MAb1 or a negative control Mouse IgG2a was added at a concentration of 10 μg/mL to 2×10^5 expression vector-transfected cells, and the resulting suspension was left standing at 4°C for 1 hour. The cells were washed once with PBS containing 5% FBS. Then, Anti-Mouse IgG FITC conjugate diluted 1000-fold with PBS containing 5% FBS was added thereto, and the resulting suspension was left standing at 4°C for 1 hour. The cells were washed with three times with PBS containing 5% FBS, then resuspended in PBS containing 5% FBS, and subjected to detection using a flow cytometer (BD FACSCalibur). Data was analyzed using Flowjo. The histogram of FITC fluorescence intensity was prepared. The antibody was determined to bind in a cross-species manner when the histogram of MAb1 was shifted to a stronger fluorescence intensity region compared with the histogram of the negative control Mouse IgG2a. The results of cross-species reactivity study demonstrated that

MAb1 did not bind to mouse ROBO4 or rat ROBO4, but bound to human ROBO4 and cynomolgus monkey ROBO4 (Figure 7).

4)-4 Binding specificity

4)-4-1 Preparation of antigen gene-expressing cell

HEK293 cells were inoculated at a concentration of 1.2×10^6 cells/dish or 1.5×10^6 cells/dish to a 60-mm dish (manufactured by Corning Inc.) and cultured overnight in a DMEM medium containing 10% FBS at 37°C under 5% CO_2 conditions. On the next day, the HEK293 cells were transfected with pCI-hROBO4, pCI-FLAG-hROBO1, pCI-hROBO2, or pCI-hROBO3 using FuGENE6 Transfection Reagent and further cultured overnight at 37°C under 5% CO_2 conditions. On the next day, the expression vector-transfected cells were treated with TrypLE Express (manufactured by Life Technologies Corp.), washed with PBS containing 5% FBS, and then suspended in PBS containing 5% FBS. The obtained cell suspension was used in flow cytometry analysis.

4)-4-2 Flow cytometry analysis

Each cell suspension prepared in 4)-4-1 was centrifuged, and the supernatant was removed. Then, MAb1, a positive control Human ROBO4 Antibody (manufactured by R&D systems, Inc.), Monoclonal ANTI-FLAG M2 antibody produced in mouse (manufactured by Sigma-Aldrich Corp.), Human ROBO2 Antibody (manufactured by R&D systems, Inc.), or Monoclonal Anti-human ROBO3 Antibody (manufactured by R&D systems, Inc.), or a negative control Mouse IgG1 or Mouse IgG2a was added at a concentration of 10 μg/mL to 2×10^5 expression vector-transfected cells, and the resulting suspension was left standing at 4°C for 1 hour. The cells were washed once with PBS containing 5% FBS. Then, Anti-Mouse IgG FITC conjugate diluted 1000-fold with PBS containing 5% FBS was added thereto, and the resulting suspension was

left standing at 4°C for 1 hour. The cells were washed with three times with PBS containing 5% FBS, then resuspended in PBS containing 5% FBS, and subjected to detection using a flow cytometer (BD FACSCalibur). Data was analyzed using Flowjo. The histogram of FITC fluorescence intensity was prepared. The antibody was determined to bind in a specific manner when the histogram of MAb1 was shifted to a stronger fluorescence intensity region compared with the histogram of the negative control Mouse IgG1 or Mouse IgG2a. The results demonstrated that MAb1 did not bind to hROBO1, hROBO2, or hROBO3 and specifically bound to hROBO4 (Figure 8). In this context, hROBO4, hROBO1, hROBO2, and hROBO3 were each confirmed to be expressed on the cell membrane using the positive control antibody (Figure 8).

4)-5 Epitope determination

4)-5-1 Preparation of antigen gene-expressing cell

HEK293 cells were inoculated at a concentration of 1.5×10^6 cells/dish to a 60-mm dish (manufactured by Corning Inc.) and cultured overnight in a DMEM medium containing 10% FBS at 37°C under 5% CO_2 conditions. On the next day, the HEK293 cells were transfected with pCI-FLAG-hROBO4-28, pCI-FLAG-hROBO4-46, pCI-FLAG-hROBO4-132, pCI-FLAG-hROBO4-210, pCI-FLAG-hROBO4-225, pCI-FLAG-hROBO4-341 using FuGENE6 Transfection Reagent and further cultured overnight at 37°C under 5% CO_2 conditions. On the next day, the expression vector-transfected cells were treated with TrypLE Express (manufactured by Life Technologies Corp.), washed with PBS containing 5% FBS, and then suspended in PBS containing 5% FBS. The obtained cell suspension was used in flow cytometry analysis.

4)-5-2 Flow cytometry analysis

Each cell suspension prepared in 4)-5-1 was centrifuged, and the supernatant was removed. Then, MAb1, a positive control Monoclonal ANTI-FLAG M2 antibody

produced in mouse, or a negative control Mouse IgG2a was added at a concentration of 10 μg/mL to 2×10^5 expression vector-transfected cells, and the resulting suspension was left standing at 4°C for 1 hour. The cells were washed once with PBS containing 5% FBS. Then, Anti-Mouse IgG FITC conjugate diluted 1000-fold with PBS containing 5% FBS was added thereto, and the resulting suspension was left standing at 4°C for 1 hour. The cells were washed with three times with PBS containing 5% FBS, then resuspended in PBS containing 5% FBS, and subjected to detection using a flow cytometer (BD FACSCalibur; BD Biosciences). Data was analyzed using Flowjo. The histogram of FITC fluorescence intensity was prepared. The antibody was determined to bind to a cell when the histogram of MAb1 was shifted to a stronger fluorescence intensity region compared with the histogram of the negative control Mouse IgG2a. The results demonstrated that MAb1 bound to the pCI-FLAG-hROBO4-28-, pCI-FLAG-hROBO4-46-, or pCI-FLAG-hROBO4-132-transfected cell and did not bind to the pCI-FLAG-hROBO4-210-, pCI-FLAG-hROBO4-225-, or pCI-FLAG-hROBO4-341-transfected cell. Thus, MAb1 was shown to recognize the amino acid sequence of Nos. 132 to 209 in human ROBO4 shown in SEQ ID NO: 2 (Figure 9). In this context, each intracellular region/domain deletion variant was confirmed to be expressed on the cell membrane using the positive control anti-FLAG antibody (Figure 9).

4)-6 Drug efficacy evaluation in monkey models with laser-induced choroidal neovascularization

4)-6-1 Anesthesia

Medetomidine hydrochloride (manufactured by Nippon Zenyaku Kogyo Co., Ltd.) was intramuscularly injected at a dose of 0.04 mg/kg to each cynomolgus monkey. 15 minutes thereafter, ketamine hydrochloride (manufactured by Daiichi Sankyo Co., Ltd.) was intramuscularly injected thereto at a dose of 15 mg/kg.

4)-6-2 Model preparation

Each cynomolgus monkey anesthetized in 4)-6-1 was retained in a monkey chair. 4% Xylocaine eye drops (manufactured by AstraZeneca plc) were applied to both eyes for anesthetic/analgesic treatment of eye surface. 5 mg/mL tropicamide-5 mg/mL phenylephrine hydrochloride mixture eye drops (manufactured by Santen Pharmaceutical Co., Ltd.) were applied to the eyes for mydriasis. The macular region of the retina was thermally damaged by laser irradiation (quantity of heat irradiated: 350-500 mW, irradiation time: 0.1 seconds, spot size: 50 μm, the number of spots: 6 or 9 spots) using a green laser photocoagulator OcuLight GLx (manufactured by Iridex Corp.).

4)-6-3 Administration of test substance

At day 7 after the model preparation, a 33G Nanopass needle was inserted into the vitreous body from the conjunctiva, and 50 μL of vehicle or 13.2 mg/mL MAb1 was injected thereto over 2 minutes using a 100-μL Hamilton syringe via a PE20 polyethylene tube. The test was conducted on each group involving 4 eyes. After completion of the administration, 0.5% levofloxacin hydrate eye drops (manufactured by Santen Pharmaceutical Co., Ltd.) were applied to the eyes.

4)-6-4 Drug efficacy evaluation

At days 7, 14, and 21 after the model preparation, the ocular fundus was photographed by a routine method using a hybrid fundus camera CX-1 (manufactured by Canon Inc.) under anesthesia. Then, 10% fluorescein was intravenously injected thereto at a dose of 0.1 mL/Kg. After completion of the intravenous injection of fluorescein, fluorescent angiography was performed every 1 minute up to 6 minutes later. The image data was stored, and the area of a site at which fluorescein

accumulated was calculated using an image analyzer (WinRoof, manufactured by Mitani Corp.). The amount of blood vessels newly formed was calculated according to the following equation: Amount of blood vessels newly formed = Area of site at which fluorescein accumulated at day 21 after model preparation - Area of site at which fluorescein accumulated at day 7 after model preparation. As a result of comparing the amount of blood vessels newly formed between the vehicle-administered group and the MAb1-administered group, three out of the four eyes in the MAb1-administered group were confirmed to decrease the amount of blood vessels newly formed, though no difference was confirmed in the amount of blood vessels newly formed between the remaining one eye and the vehicle group. This means that the administration of MAb1 suppressed laser-induced choroidal neovascularization (Figure 10).

(Example 5) Cloning and sequencing of MAb1 cDNA

5)-1 Determination of N-terminal amino acid sequences of MAb1 heavy and light chains

In order to determine the N-terminal amino acid sequences of the heavy and light chains of MAb1, MAb1 purified in Example 2)-8 was separated by SDS-PAGE. The proteins thus separated in the gel were transferred from the gel to a Sequi-Blot PVDF membrane (Bio-Rad Laboratories, Inc.), which was in turn washed with a washing buffer (25 mM NaCl, 10 mM sodium borate buffer, pH 8.0), then stained by dipping for 5 minutes in a staining solution (50% methanol, 20% acetic acid, 0.05% Coomassie Brilliant Blue), and then decolorized with 90% methanol. Band portions corresponding to the heavy chain (band with smaller mobility) and the light chain (band with larger mobility) visualized on the PVDF membrane were cleaved off. Their respective N-terminal amino acid sequences were identified according to the automatic Edman method (see Edman et al., (1967) Eur. J. Biochem. 1, 80) using Procise cLC protein sequencer Model 492cLC (Applied Biosystems, Inc.). As a result, the N-

terminal amino acid sequence of the band corresponding to the heavy chain of MAb1 was

EVQLVESGGGLVKPGGSLKL, and

the N-terminal amino acid sequence of the band corresponding to the light chain was DAVMTQTPLSLPVSL.

5)-2 Preparation of mRNA from MAb1-producing hybridoma

In order to clone heavy chain- and light chain-encoding cDNAs of MAb1, mRNA was prepared from the MAb1-producing hybridoma using mRNA Isolation kit (Roche Applied Science).

5)-3 Cloning and sequencing of MAb1 cDNA

Several oligonucleotide primers hybridizing to the 5'-terminal sequence of the coding region of the antibody gene and the stop codon-containing 3'-terminal sequence thereof, respectively, were synthesized with reference to the N-terminal amino acid sequences of the heavy and light chains determined based on the γ1 and κ isotypes of the MAb1 heavy and light chains, respectively (Examples 2)-7) and 5-1), and the antibody amino acid sequence database prepared by Kabat et al. (see Strausberg, R. L., et al. (2002) Proc. Natl. Acad. Sci. U. S. A. 99. 16899-16903, and Kabat, E. A., et al. (1991) in Sequences of Proteins of Immunological Interest Vol. I and II, U. S. Department of Health and Human Services). Heavy chain- and light chain-encoding cDNAs were amplified using the mRNA prepared in 5)-2 and TaKaRa One Step RNA PCR Kit (AMV) (Takara Bio Inc.). As a result, the heavy chain- and light chain-encoding cDNAs of the antibody were successfully amplified using the following primer set:

primer set for the heavy chain

LYHF6: 5'-cctcaccatgaactttgg-3'

G1EVR1: 5'-aagatatcttatttaccaggagagtgggagag-3'

primer set for the light chain

MK19EIF1: 5'-aagaattcatgaagttgcctgttagg-3'

KEVR1: 5'-aagatatcttaacactcattcctgttgaagct-3'

The heavy and light chain cDNAs amplified by PCR were separately cloned using pEF6/V5-His TOPO TA Expression Kit (Invitrogen Corp.). The nucleotide sequences of the cloned cDNAs encoding the respective variable regions of the heavy and light chains were determined using a gene sequencer ("ABI PRISM 3700 DNA Analyzer; Applied Biosystems" or "Applied Biosystems 3730xl Analyzer; Applied Biosystems"). The sequencing reaction was performed using GeneAmp 9700 (Applied Biosystems, Inc.).

The determined nucleotide sequence of cDNA encoding the heavy chain variable region of MAb1 is shown in SEQ ID NO: 30 , and its amino acid sequence is shown in SEQ ID NO: 31. The nucleotide sequence of cDNA encoding the light chain variable region of the mouse antibody MAb1 is shown in SEQ ID NO: 32 , and its amino acid sequence is shown in SEQ ID NO: 33.

(Example 6) Preparation of chimeric MAb1 (cMAb1)

6)-1 Preparation of expression vectors pCMA-LK, pCMA-G1, and pCMA-G2

6)-1-1 Construction of chimeric and humanized light chain expression vector pCMA-LK

A plasmid pcDNA3.3-TOPO/LacZ (Invitrogen Corp.) was digested with restriction enzymes XbaI and PmeI, and the obtained fragment of approximately 5.4 kb was ligated to a DNA fragment comprising a DNA sequence (SEQ ID NO: 34) encoding a human κ chain secretory signal and a human κ chain constant region using In-Fusion Advantage PCR cloning kit (Clontech Laboratories, Inc.) to prepare pcDNA3.3/LK.

PCR was performed with pcDNA3.3/LK as a template using a primer set shown below. The obtained fragment of approximately 3.8 kb was phosphorylated and then self-ligated to construct, from pcDNA3.3/LK, a chimeric and humanized light chain expression vector pCMA-LK having a signal sequence-encoding sequence, a cloning site, and a human κ chain constant region-encoding sequence downstream of a CMV promoter.

Primer set

3. 3-F1: 5'-tataccgtcgacctctagctagagcttggc-3'

3. 3-R1: 5'-gctatggcagggcctgccgccccgacgttg-3'

6)-1-2 Construction of chimeric and humanized IgG1-type heavy chain expression vector pCMA-G1

pCMA-LK was digested with XbaI and PmeI to remove a sequence encoding a κ chain secretory signal and a human κ chain constant region. The resulting DNA fragment was ligated to a DNA fragment comprising a DNA sequence (SEQ ID NO: 35) encoding the amino acids of a human heavy chain signal sequence and a human IgG1 constant region using In-Fusion Advantage PCR cloning kit (Clontech Laboratories, Inc.) to construct a chimeric and humanized IgG1-type heavy chain expression vector pCMA-G1 having a signal sequence-encoding sequence, a cloning site, and a human IgG1 heavy chain constant region-encoding sequence downstream of a CMV promoter.

6)-1-3 Construction of chimeric and humanized IgG2-type heavy chain expression vector pCMA-G2

pCMA-LK was digested with XbaI and PmeI to remove a sequence encoding a κ chain secretory signal and a human κ chain constant region. The resulting DNA fragment was ligated to a DNA fragment comprising a DNA sequence (SEQ ID NO: 36) encoding the amino acids of a human heavy chain signal sequence and a human

IgG2 constant region using In-Fusion Advantage PCR cloning kit (Clontech Laboratories, Inc.) to construct a chimeric and humanized IgG2-type heavy chain expression vector pCMA-G2 having a signal sequence-encoding sequence, a cloning site, and a human IgG2 heavy chain constant region-encoding sequence downstream of a CMV promoter.

6)-2 Construction of chimeric MAb1 light chain expression vector

A site comprising cDNA encoding the light chain variable region was amplified with cDNA encoding the light chain variable region of MAb1 as a template using KOD-Plus- (TOYOBO CO., LTD.) and a primer set shown below, and inserted into a restriction enzyme BsiWI-cleaved site of the general-purpose vector pCMA-LK for chimeric and humanized antibody light chain expression using In-Fusion Advantage PCR cloning kit (Clontech Laboratories, Inc.) to construct a chimeric MAb1 light chain expression vector. The obtained expression vector was designated as "pCMA-LK/MAb1 L". The nucleotide sequence encoding the chimeric MAb1 light chain is shown in SEQ ID NO: 37 , and its amino acid sequence is shown in SEQ ID NO: 38. Nucleotide Nos. 1 to 60 of SEQ ID NO: 37 represent the nucleotide sequence encoding the signal sequence. Nucleotide Nos. 61 to 402 thereof represent the nucleotide sequence encoding the variable region. Nucleotide Nos. 403 to 717 thereof represent the nucleotide sequence encoding the constant region. Amino acid Nos. 1 to 20 of SEQ ID NO: 38 represent the amino acids of the signal sequence. Amino acid Nos. 21 to 134 thereof represent the amino acids of the variable region. Amino acid Nos. 135 to 239 represent the amino acids of the constant region.

Primer set for the light chain

MAb1 LF: 5'-tctccggcgcgtacggcgatgctgtgatgacccaaactccactctcc-3'

MAb1 LR: 5'-ggaggggggcggccacagcccgtttgatttccagcttggtgcctcc-3'

6)-3 Construction of chimeric MAb1 IgG1-type heavy chain expression vector

A site comprising cDNA encoding the heavy chain variable region was amplified with cDNA encoding the heavy chain variable region of MAb1 as a template using KOD-Plus- (TOYOBO CO., LTD.) and a primer set shown below, and inserted into a restriction enzyme BsiWI-cleaved site of the chimeric and humanized IgG1-type heavy chain expression vector pCMA-G1 using In-Fusion Advantage PCR cloning kit (Clontech Laboratories, Inc.) to construct a chimeric MAb1 IgG1-type heavy chain expression vector. The obtained expression vector was designated as "pCMA-G1/ MAb1 H". The nucleotide sequence encoding the chimeric MAb1 IgG1-type heavy chain is shown in SEQ ID NO: 39 , and its amino acid sequence is shown in SEQ ID NO: 40. Nucleotide Nos. 1 to 57 of SEQ ID NO: 39 represent the nucleotide sequence encoding the signal sequence. Nucleotide Nos. 58 to 411 thereof represent the nucleotide sequence encoding the variable region. Nucleotide Nos. 412 to 1401 thereof represent the nucleotide sequence encoding the constant region. Amino acid Nos. 1 to 19 of SEQ ID NO: 40 represent the amino acid sequence of the signal sequence. Amino acid Nos. 20 to 137 thereof represent the amino acid sequence of the variable region. Amino acid Nos. 138 to 467 represent the amino acid sequence of the constant region.

Primer set for the IgG1-type heavy chain

MAb1 HF: 5'-cagatgggtgctgagcgaagtgcagctggtggagtctgggggag-3'

MAb1 H1R: 5'-ttggtggaggctgagctgactgtgagagtggtgccgtggccccag-3'

6)-4 Construction of chimeric MAb1 IgG2-type heavy chain expression vector

A site comprising cDNA encoding the heavy chain variable region was amplified with cDNA encoding the heavy chain variable region of MAb1 as a template using KOD-Plus- (TOYOBO CO., LTD.) and a primer set shown below, and inserted into a restriction enzyme BsiWI-cleaved site of the chimeric and humanized IgG2-type heavy

chain expression vector pCMA-G2 using In-Fusion Advantage PCR cloning kit (Clontech Laboratories, Inc.) to construct a chimeric MAb1 IgG2-type heavy chain expression vector. The obtained expression vector was designated as "pCMA-G2/MAb1". The nucleotide sequence encoding the chimeric MAb1 IgG2-type heavy chain is shown in SEQ ID NO: 41 , and its amino acid sequence is shown in SEQ ID NO: 42.

Primer set for the IgG2-type heavy chain

MAb1 HF: 5'-cagatgggtgctgagcgaagtgcagctggtggagtctgggggag-3'

MAb1 2R: 5'-ttggtgctggctgagctgactgtgagagtggtgccgtggccccag-3'

(Example 7) Preparation of IgG1-type chimeric MAb1 antibody and IgG2-type chimeric MAb1 antibody

7)-1 Production of IgG1-type chimeric MAb1 antibody and IgG2-type chimeric MAb1 antibody

FreeStyle 293-F cells (Invitrogen Corp.) were subcultured and cultured according to the manual. 1.2×10^9 cells of FreeStyle 293-F cells (Invitrogen Corp.) at the logarithmic growth phase were inoculated to a 3L Fernbach Erlenmeyer Flask (Corning Inc.), diluted with FreeStyle 293 expression medium (Invitrogen Corp.) into 1.0×10^6 cells/mL, and then shake-cultured in an 8% CO_2 incubator at 90 rpm at 37°C for 1 hour. 3.6 mg of polyethyleneimine (Polysciences, Inc., #24765) was dissolved in 20 mL of Opti-Pro SFM medium (Invitrogen Corp.). Next, an H chain expression vector (0.4 mg) and an L chain expression vector (0.8 mg) prepared using PureLink HiPure Plasmid kit (Invitrogen Corp.) were suspended in 20 mL of Opti-Pro SFM medium (Invitrogen Corp.). 20 mL of the expression vector/Opti-Pro SFM mixture solution was added to 20 mL of the polyethyleneimine/Opti-Pro SFM mixture solution, and the mixture was gradually stirred, further left for 5 minutes, and then added to the FreeStyle 293-F cells. The cells were shake-cultured in an 8% CO_2 incubator at 90

rpm at 37°C for 7 days, and the obtained culture supernatant was filtered through Disposable Capsule Filter (Advantec, #CCS-045-E1H).

IgG1-type and IgG2-type chimeric MAb1 antibodies obtained by the combinations between pCMA-G1/MAb1 H and pCMA-LK/MAb1 L and between pCMA-G2/MAb1 H and pCMA-LK/MAb1 L are abbreviated to "cMAb1-1" and "cMAb1-2", respectively. The term "cMAb1" means both IgG1-type and IgG2-type chimeric MAb1 antibodies.

7)-2 Purification of cMAb1-1 and cMAb1-2

The culture supernatant obtained above in 7-1) was purified by rProtein A affinity chromatography (at 4-6°C). A buffer replacement step after the rProtein A affinity chromatography purification was carried out at room temperature. First, 1100 to 1200 ml of the culture supernatant was applied to MabSelect SuRe (manufactured by GE Healthcare Bio-Sciences Crop., HiTrap column; volume 1 mL × 2 connected) equilibrated with PBS. The whole culture solution was placed in the column, and the column was then washed with PBS. Next, elution was performed with a 2 M arginine hydrochloride solution (pH 4.0) to collect an antibody-containing fraction. The buffer in this fraction was replaced by PBS using a desalting column (manufactured by GE Healthcare Bio-Sciences Corp., HiTrap Desalting column; volume 5 mL × 2 connected). Finally, the fraction was concentrated with Centrifugal UF Filter Device VIVASPIN20 (molecular weight cutoff: 30 K, Sartorius, at 4°C) into an IgG concentration of 20.0 mg/mL or higher and used as a purification sample.

7)-3 Properties of cMAb1-1 and cMAb1-2

7)-3-1 Activation of ROBO4 downstream signal

This test was conducted according to the method of 4)-1 except that: Human IgG (manufactured by Sigma-Aldrich Corp.) was used as a negative control; and Human

IgG, cMAb1-1, or cMAb1-2 was added at a final concentration of 0.313 μg/mL to a medium. As a result, the negative control Human IgG did not influence the IL-8 promoter activity in the cells transiently expressing human ROBO4, whereas cMAb1-1 and cMAb1-2 increased the IL-8 promoter activity (Figure 11). These results demonstrated that cMAb1-1 or cMAb1-2 activated the downstream signal of ROBO4, as in MAb1.

7)-3-2 HUVEC migration test

This test was conducted according to the method of 4)-2 except that: Human IgG was used as a negative control; and Human IgG, cMAb1-1, or cMAb1-2 was added at a final concentration of 0.5 μg/mL to a medium. As a result, cMAb1-1 or cMAb1-2 suppressed the migration of HUVEC induced by bFGF (Figure 12). These results demonstrated that cMAb1-1 or cMAb1-2 suppressed the migration of HUVEC, as in MAb1.

(Example 8) Design of humanized antibody of mouse anti-human ROBO4 monoclonal antibody MAb1

8)-1 Design of humanized version of MAb1

8)-1-1 Molecular modeling of MAb1 variable region

The molecular modeling of the MAb1 variable regions was carried out by a generally known homology modeling method (Methods in Enzymology, 203, 121-153, (1991)). The primary sequences (available as three-dimensional structures induced from x-ray crystal structures) of human immunoglobulin variable regions registered in Protein Data Bank (Nuc. Acid Res. 35, D301-D303 (2007)) were compared with those of the MAb1 variable regions thus determined. As a result, 3FFD was selected because of its highest sequence homology to the MAb1 heavy chain variable region among antibodies deficient in framework. Also, 1T66 was selected because of its

highest sequence homology to the MAb1 light chain variable region. The three-dimensional structures of the framework regions were prepared by obtaining a "framework model" from the combination of the coordinates of 3FFD and 1T66 corresponding to the MAb1 heavy and light chains. As for the CDRs of MAb1, CDRL1, CDRL2, CDRL3, CDRH1, and CDRH2 were assigned to clusters 16A, 7A, 9A, 10A, and 10B, respectively, according to the classification of Thornton et al. (J. Mol. Biol., 263, 800-815, (1996)). CDRH3 was classified into k(7)B using the H3 rule (FEBS letter 399, 1-8 (1996)). Subsequently, the typical conformation of each CDR was incorporated into the framework model.

Finally, energy calculation for excluding disadvantageous interatomic contact was conducted to obtain a molecular model likely to be the MAb1 variable regions in terms of energy. These procedures were performed using a commercially available protein three-dimensional structure prediction program Prime and coordinate searching program MacroModel (Schrodinger, LLC).

8)-1-2 Design of humanized MAb1 amino acid sequence

Each humanized MAb1 antibody (hMAb1) was constructed by a generally known CDR grafting method (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). Two different acceptor antibodies were selected on the basis of the homology of amino acids in framework regions. The sequences of the MAb1 framework regions were compared with all human frameworks in the Kabat antibody amino acid sequence database (Nuc. Acid Res. 29, 205-206 (2001)). As a result, a B3 antibody was selected as an acceptor because of its 83% sequence homology to the framework regions. The amino acid residues of the B3 framework regions were aligned with those of MAb1 to identify the positions of different amino acids used. These positions of the residues were analyzed using the three-dimensional model of MAb1 constructed above. Then, donor residues to be grafted onto the acceptor were selected according to the criteria

given by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). Some selected donor residues were integrated into the acceptor antibody to construct the sequence of humanized MAb1 as described in Examples below. Also, hMAb1 variants were constructed by the substitution of 1 to 3 amino acid residue(s) in each CDR of hMAb1 with other amino acid residues, as described in Examples below.

8)-2 Humanization of cMAb1 heavy chain

8)-2-1 hMAb1-H1-type heavy chain:

A humanized MAb1 heavy chain designed by replacing amino acid No. 32 (lysine) with glutamine, amino acid No. 38 (lysine) with arginine, amino acid No. 59 (threonine) with alanine, amino acid No. 61 (glutamic acid) with glycine, amino acid No. 63 (arginine) with glycine, amino acid No. 95 (glutamic acid) with lysine, amino acid No. 103 (serine) with asparagine, amino acid No. 107 (serine) with alanine, amino acid No. 112 (methionine) with valine, amino acid No. 114 (phenylalanine) with tyrosine, amino acid No. 129 (histidine) with glutamine, amino acid No. 132 (threonine) with leucine, and amino acid No. 133 (leucine) with valine, in the cMAb1-2 heavy chain represented by SEQ ID NO: 42 was designated as a "hMAb1-H1-type heavy chain".

The amino acid sequence of the hMAb1-H1-type heavy chain is shown in SEQ ID NO: 56 . A sequence consisting of amino acid residues 1 to 19, a sequence consisting of amino acid residues 20 to 137, and a sequence consisting of amino acid residues 138 to 463 in the amino acid sequence of SEQ ID NO: 56 correspond to a signal sequence, a heavy chain variable region, and a heavy chain constant region, respectively. A sequence consisting of amino acid residues 50 to 54, a sequence consisting of amino acid residues 69 to 85, and a sequence consisting of amino acid residues 118 to 126 in the amino acid sequence of SEQ ID NO: 56 correspond to a CDRH1 sequence, a CDRH2 sequence, and a CDRH3 sequence, respectively. A

nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 56 is shown in SEQ ID NO: 55. A sequence consisting of nucleotides 1 to 57, a sequence consisting of nucleotides 58 to 411, and a sequence consisting of nucleotides 412 to 1389 in the nucleotide sequence of SEQ ID NO: 55 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 55 and the amino acid sequence of SEQ ID NO: 56 are also shown in Figures 31 and 32, respectively.

8)-2-2 hMAb1-H2-type heavy chain:

A humanized MAb1 heavy chain designed by replacing amino acid No. 32 (lysine) with glutamine, amino acid No. 38 (lysine) with arginine, amino acid No. 59 (threonine) with alanine, amino acid No. 61 (glutamic acid) with glycine, amino acid No. 63 (arginine) with glycine, amino acid No. 72 (asparagine) with glutamine, amino acid No. 95 (glutamic acid) with lysine, amino acid No. 103 (serine) with asparagine, amino acid No. 107 (serine) with alanine, amino acid No. 112 (methionine) with valine, amino acid No. 114 (phenylalanine) with tyrosine, amino acid No. 129 (histidine) with glutamine, amino acid No. 132 (threonine) with leucine, and amino acid No. 133 (leucine) with valine, in the cMAb1-2 heavy chain represented by SEQ ID NO: 42 was designated as a "hMAb1-H2-type heavy chain".

The amino acid sequence of the hMAb1-H2-type heavy chain is shown in SEQ ID NO: 58 . A sequence consisting of amino acid residues 1 to 19, a sequence consisting of amino acid residues 20 to 137, and a sequence consisting of amino acid residues 138 to 463 in the amino acid sequence of SEQ ID NO: 58 correspond to a signal sequence, a heavy chain variable region, and a heavy chain constant region, respectively. A sequence consisting of amino acid residues 50 to 54, a sequence consisting of amino acid residues 69 to 85, and a sequence consisting of amino acid residues 118 to 126 in the amino acid sequence of SEQ ID NO: 58 correspond to a

CDRH1 sequence, a CDRH2 sequence, and a CDRH3 sequence, respectively. A nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 58 is shown in SEQ ID NO: 57 . A sequence consisting of nucleotides 1 to 57, a sequence consisting of nucleotides 58 to 411, and a sequence consisting of nucleotides 412 to 1389 in the nucleotide sequence of SEQ ID NO: 57 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 57 and the amino acid sequence of SEQ ID NO: 58 are also shown in Figures 33 and 34, respectively.

8)-2-3 hMAb1-H3-type heavy chain:

A humanized MAb1 heavy chain designed by replacing amino acid No. 32 (lysine) with glutamine, amino acid No. 38 (lysine) with arginine, amino acid No. 59 (threonine) with alanine, amino acid No. 61 (glutamic acid) with glycine, amino acid No. 95 (glutamic acid) with lysine, amino acid No. 103 (serine) with asparagine, amino acid No. 107 (serine) with alanine, amino acid No. 112 (methionine) with valine, amino acid No. 114 (phenylalanine) with tyrosine, amino acid No. 129 (histidine) with glutamine, amino acid No. 132 (threonine) with leucine, and amino acid No. 133 (leucine) with valine, in the cMAb1-2 heavy chain represented by SEQ ID NO: 42 was designated as a "hMAb1-H3-type heavy chain".

The amino acid sequence of the hMAb1-H3-type heavy chain is shown in SEQ ID NO: 60. A sequence consisting of amino acid residues 1 to 19, a sequence consisting of amino acid residues 20 to 137, and a sequence consisting of amino acid residues 138 to 463 in the amino acid sequence of SEQ ID NO: 60 correspond to a signal sequence, a heavy chain variable region, and a heavy chain constant region, respectively. A sequence consisting of amino acid residues 50 to 54, a sequence consisting of amino acid residues 69 to 85, and a sequence consisting of amino acid residues 118 to 126 in the amino acid sequence of SEQ ID NO: 60 correspond to a

CDRH1 sequence, a CDRH2 sequence, and a CDRH3 sequence, respectively. A nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 60 is shown in SEQ ID NO: 59. A sequence consisting of nucleotides 1 to 57, a sequence consisting of nucleotides 58 to 411, and a sequence consisting of nucleotides 412 to 1389 in the nucleotide sequence of SEQ ID NO: 59 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 59 and the amino acid sequence of SEQ ID NO: 60 are also shown in Figures 35 and 36, respectively.

8)-2-4 hMAb1-H4-type heavy chain:

A humanized MAb1 heavy chain designed by replacing amino acid No. 32 (lysine) with glutamine, amino acid No. 38 (lysine) with arginine, amino acid No. 59 (threonine) with alanine, amino acid No. 61 (glutamic acid) with glycine, amino acid No. 72 (asparagine) with glutamine, amino acid No. 95 (glutamic acid) with lysine, amino acid No. 103 (serine) with asparagine, amino acid No. 107 (serine) with alanine, amino acid No. 112 (methionine) with valine, amino acid No. 114 (phenylalanine) with tyrosine, amino acid No. 129 (histidine) with glutamine, amino acid No. 132 (threonine) with leucine, and amino acid No. 133 (leucine) with valine, in the cMAb1-2 heavy chain represented by SEQ ID NO: 42 was designated as a "hMAb1-H4-type heavy chain".

The amino acid sequence of the hMAb1-H4-type heavy chain is shown in SEQ ID NO: 62 . A sequence consisting of amino acid residues 1 to 19, a sequence consisting of amino acid residues 20 to 137, and a sequence consisting of amino acid residues 138 to 463 in the amino acid sequence of SEQ ID NO: 62 correspond to a signal sequence, a heavy chain variable region, and a heavy chain constant region, respectively. A sequence consisting of amino

acid residues 50 to 54, a sequence consisting of amino acid residues 69 to 85, and a sequence consisting of amino acid residues 118 to 126 in the amino acid sequence of SEQ ID NO: 62 correspond to a CDRH1 sequence, a CDRH2 sequence, and a CDRH3 sequence, respectively. A nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 62 is shown in SEQ ID NO: 61 . A sequence consisting of nucleotides 1 to 57, a sequence consisting of nucleotides 58 to 411, and a sequence consisting of nucleotides 412 to 1389 in the nucleotide sequence of SEQ ID NO: 61 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 61 and the amino acid sequence of SEQ ID NO: 62 are also shown in Figures 37 and 38, respectively.

8)-3 Humanization of MAb1 light chain

8)-3-1 hMAb1-L1-type light chain:

A humanized MAb1 light chain designed by replacing amino acid No. 22 (alanine) with isoleucine, amino acid No. 27 (threonine) with serine, amino acid No. 34 (serine) with threonine, amino acid No. 37 (aspartic acid) with glutamic acid, amino acid No. 38 (glutamine) with proline, amino acid No. 62 (phenylalanine) with leucine, amino acid No. 84 (leucine) with proline, amino acid No. 108 (phenylalanine) with valine, amino acid No. 112 (phenylalanine) with tyrosine, amino acid No. 125 (glycine) with proline, amino acid No. 129 (leucine) with valine, amino acid No. 130 (glutamic acid) with aspartic acid, and amino acid No. 134 (alanine) with threonine, in the cMAb1 light chain represented by SEQ ID NO: 38 was designated as a "hMAb1-L1-type light chain".

The amino acid sequence of the hMAb1-L1-type light chain is shown in SEQ ID NO: 64. A sequence consisting of amino acid residues 1 to 20, a sequence consisting of amino acid residues 21 to 134, and a sequence consisting of amino acid residues 135

to 239 in the amino acid sequence of SEQ ID NO: 64 correspond to a signal sequence, a light chain variable region, and a light chain constant region, respectively. A sequence consisting of amino acid residues 44 to 59, a sequence consisting of amino acid residues 75 to 81, and a sequence consisting of amino acid residues 114 to 122 in the amino acid sequence of SEQ ID NO: 64 correspond to a CDRL1 sequence, a CDRL2 sequence, and a CDRL3 sequence, respectively. A nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 64 is shown in SEQ ID NO: 63 . A sequence consisting of nucleotides 1 to 60, a sequence consisting of nucleotides 61 to 402, and a sequence consisting of nucleotides 403 to 717 in the nucleotide sequence of SEQ ID NO: 63 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 63 and the amino acid sequence of SEQ ID NO: 64 are also shown in Figures 39 and 40, respectively.

8)-3-2 hMAb1-L2-type light chain:

A humanized MAb1 light chain designed by replacing amino acid No. 22 (alanine) with isoleucine, amino acid No. 27 (threonine) with serine, amino acid No. 34 (serine) with threonine, amino acid No. 37 (aspartic acid) with glutamic acid, amino acid No. 38 (glutamine) with proline, amino acid No. 52 (serine) with glutamic acid, amino acid No. 54 (glycine) with lysine, amino acid No. 56 (threonine) with leucine, amino acid No. 62 (phenylalanine) with leucine, amino acid No. 84 (leucine) with proline, amino acid No. 108 (phenylalanine) with valine, amino acid No. 112 (phenylalanine) with tyrosine, amino acid No. 125 (glycine) with proline, amino acid No. 129 (leucine) with valine, amino acid No. 130 (glutamic acid) with aspartic acid, and amino acid No. 134 (alanine) with threonine, in the cMAb1 light chain represented by SEQ ID NO: 38 was designated as a "hMAb1-L2-type light chain".

The amino acid sequence of the hMAb1-L2-type light chain is shown in SEQ ID NO: 66 . A sequence consisting of amino acid residues 1 to 20, a sequence consisting of amino acid residues 21 to 134, and a sequence consisting of amino acid residues 135 to 239 in the amino acid sequence of SEQ ID NO: 66 correspond to a signal sequence, a light chain variable region, and a light chain constant region, respectively. A sequence consisting of amino acid residues 44 to 59, a sequence consisting of amino acid residues 75 to 81, and a sequence consisting of amino acid residues 114 to 122 in the amino acid sequence of SEQ ID NO: 66 correspond to a CDRL1 sequence, a CDRL2 sequence, and a CDRL3 sequence, respectively. A nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 66 is shown in SEQ ID NO: 65. A sequence consisting of nucleotides 1 to 60, a sequence consisting of nucleotides 61 to 402, and a sequence consisting of nucleotides 403 to 717 in the nucleotide sequence of SEQ ID NO: 65 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 65 and the amino acid sequence of SEQ ID NO: 66 are also shown in Figures 41 and 42, respectively.

(Example 9) Preparation of humanized MAb1

9)-1 Construction of humanized MAb1 heavy chain expression vector

9)-1-1 Construction of hMAb1-H1-type heavy chain expression vector

DNA comprising the gene encoding the hMAb1-H1-type heavy chain variable region represented by amino acid Nos. 20 to 137 of SEQ ID NO: 56 was synthesized (GeneArt artificial gene synthesis service) and cleaved with a restriction enzyme BlpI. The obtained DNA fragment was inserted to a restriction enzyme BlpI-cleaved site of the chimeric and humanized IgG2-type heavy chain expression vector (pCMA-G2) to construct a hMAb1-H1-type heavy chain expression vector. The obtained expression

vector was designated as "pCMA-G2/hMAb1-H1". The nucleotide sequence of the hMAb1-H1-type heavy chain is shown in SEQ ID NO: 55 .

9)-1-2 Construction of hMAb1-H2-type heavy chain expression vector

A hMAb1-H2-type heavy chain expression vector was constructed with the pCMA-G2/hMAb1-H1 constructed in 9)-1-1 as a template using a primer set shown below and QuikChange XL Site-Directed Mutagenesis Kit (Agilent Technologies, Inc.). The obtained expression vector was designated as "pCMA-G1/hMAb1-H2". The nucleotide sequence of the hMAb1-H2-type heavy chain is shown in SEQ ID NO: 57 , and its amino acid sequence is shown in SEQ ID NO: 58.

Primer set:

H-N53Q-F: 5'-gggtggcaaccatcagccaaggcggcacctacacctac-3'

H-N53Q-R: 5'-gtaggtgtaggtgccgccttggctgatggttgccaccc-3'

9)-1-3 Construction of hMAb1-H3-type heavy chain expression vector

DNA comprising the gene encoding the hMAb1-H3-type heavy chain variable region represented by amino acid Nos. 20 to 137 of SEQ ID NO: 60 was synthesized (GeneArt artificial gene synthesis service) and cleaved with a restriction enzyme BlpI. The obtained DNA fragment was inserted to a restriction enzyme BlpI-cleaved site of the chimeric and humanized IgG2-type heavy chain expression vector (pCMA-G2) to construct a hMAb1-H3-type heavy chain expression vector. The obtained expression vector was designated as "pCMA-G2/hMAb1-H3". The nucleotide sequence of the hMAb1-H3-type heavy chain is shown in SEQ ID NO: 59 .

9)-1-4 Construction of hMAb1-H4-type heavy chain expression vector

A hMAb1-H4-type heavy chain expression vector was constructed with the pCMA-G2/hMAb1-H3 constructed in 9)-1-3 as a template using a primer set shown

below and QuikChange XL Site-Directed Mutagenesis Kit (Agilent Technologies, Inc.). The obtained expression vector was designated as "pCMA-G1/hMAb1-H4". The nucleotide sequence of the hMAb1-H4-type heavy chain is shown in SEQ ID NO: 61 , and its amino acid sequence is shown in SEQ ID NO: 62.

Primer set:

H-N53Q-F: 5'-gggtggcaaccatcagccaaggcggcacctacacctac-3'

H-N53Q-R: 5'-gtaggtgtaggtgccgccttggctgatggttgccaccc-3'

9)-2 Construction of humanized MAb1 light chain expression vector

9)-2-1 Construction of hMAb1-L1-type light chain expression vector

DNA comprising the gene encoding the hMAb1-L1-type light chain variable region represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 was synthesized (GeneArt artificial gene synthesis service) and cleaved with a restriction enzyme BsiWI. The obtained DNA fragment was inserted to a restriction enzyme BsiWI-cleaved site of the general-purpose vector (pCMA-LK) for chimeric and humanized antibody light chain expression to construct a hMAb1-L1-type light chain expression vector. The obtained expression vector was designated as "pCMA-LK/hMAb1-L1". The nucleotide sequence of the hMAb1-L1-type light chain is shown in SEQ ID NO: 63 .

9)-2-2 Construction of hMAb1-L2-type light chain expression vector

DNA fragments were obtained by PCR with the pCMA-LK/hMAb1-L1 constructed in 9)-2-1 as a template using KOD-Plus- (TOYOBO CO., LTD.) and each of primer sets A and B, and linked by overlap extension PCR using primer set C to prepare DNA comprising a gene encoding the hMAb1-L2-type light chain. This DNA was cleaved with restriction enzymes XbaI and PmeI to obtain a DNA fragment, which was then inserted to a restriction enzyme XbaI/PmeI-cleaved site of the general-purpose vector (pCMA-LK) for chimeric and humanized antibody light chain expression to

construct a hMAb1-L2-type light chain expression vector. The obtained expression vector was designated as "pCMA-LK/hMAb1-L2". The nucleotide sequence of the hMAb1-L2-type light chain is shown in SEQ ID NO: 65 , and its amino acid sequence is shown in SEQ ID NO: 66.

Primer set A

L inf-F: 5'-gcctccggactctagagccaccatggtgctgcagacccaggtgttc-3'

L-EKL-R: 5'-caggtacaggttcttgttctcgttttccaggctctggctgcttctgcagc-3'

Primer set B

L-EKL-F: 5'-gaaaacgagaacaagaacctgtacctgaactggtatctgcagaagcccg-3'

L inf-R: 5'-tagcctcccccgtttaaacgggcccctaacactcccccctg-3'

Primer set C

L inf-F: 5'-gcctccggactctagagccaccatggtgctgcagacccaggtgttc-3'

L inf-R: 5'-tagcctcccccgtttaaacgggcccctaacactcccccctg-3'

(Example 10) Preparation of humanized MAb1

10-7-1) Production of humanized MAb1

FreeStyle 293-F cells (Invitrogen Corp.) were subcultured and cultured according to the manual.

1.2×10^9 cells of FreeStyle 293-F cells (Invitrogen Corp.) at the logarithmic growth phase were inoculated to 3L Fernbach Erlenmeyer Flask (Corning Inc.), diluted with FreeStyle 293 expression medium (Invitrogen Corp.) into 1.0×10^6 cells/mL, and then shake-cultured in an 8% CO_2 incubator at 90 rpm at 37°C for 1 hour. 3.6 mg of polyethyleneimine (Polysciences, Inc., #24765) was dissolved in 20 mL of Opti-Pro SFM medium (Invitrogen Corp.). Next, an H chain expression vector (0.4 mg) and an

L chain expression vector (0.8 mg) prepared using PureLink HiPure Plasmid kit (Invitrogen Corp.) were suspended in 20 mL of Opti-Pro SFM medium (Invitrogen Corp.). 20 mL of the expression vector/Opti-Pro SFM mixture solution was added to 20 mL of the polyethyleneimine/Opti-Pro SFM mixture solution, and the mixture was gradually stirred, further left for 5 minutes, and then added to the FreeStyle 293-F cells. The cells were shake-cultured in an 8% CO_2 incubator at 90 rpm at 37°C for 7 days, and the obtained culture supernatant was filtered through Disposable Capsule Filter (Advantec, #CCS-045-E1H), and purified in the same way as in 7)-2.

The humanized MAb1 antibody obtained by the combination between pCMA-G2/hMAb1-H1 and pCMA-LK/hMAb1-L1 was designated as "H-1040". The humanized MAb1 antibody obtained by the combination between pCMA-G2/hMAb1-H2 and pCMA-LK/hMAb1-L1 was designated as "H-1140". The humanized MAb1 antibody obtained by the combination between pCMA-G2/hMAb1-H2 and pCMA-LK/hMAb1-L2 was designated as "H-1143". The humanized MAb1 antibody obtained by the combination between pCMA-G2/hMAb1-H3 and pCMA-LK/hMAb1-L1 was designated as "H-2040". The humanized MAb1 antibody obtained by the combination between pCMA-G2/hMAb1-H4 and pCMA-LK/hMAb1-L1 was designated as "H-2140". The humanized MAb1 antibody obtained by the combination between pCMA-G2/hMAb1-H4 and pCMA-LK/hMAb1-L2 was designated as "H-2143".

(Example 11) Property of anti-ROBO4 humanized antibody

11)-1 Binding affinity

The dissociation constant between each antibody and rROBO4-ECD was determined with the antibody immobilized as a ligand and the antigen as an analyte using Biacore 3000 (manufactured by GE Healthcare Bio-Sciences Corp.). Approximately 80 RU of the antibody was bound via an anti-human IgG antibody

(manufactured by GE Healthcare Bio-Sciences Corp.) immobilized on a sensor chip CM5 (manufactured by GE Healthcare Bio-Sciences Corp.) by an amine coupling method. The running buffer used was PBS containing 0.05% Surfactant P20. Dilution series solutions (0.1-200 nM) of the antigen were added at a flow rate of 30 μL/min. for 300 seconds onto the antibody-bound chip. Subsequently, a dissociation phase was monitored for 300 seconds. 3 M $MgCl_2$ was added thereto as a regeneration solution at a flow rate of 10 μL/min. for 30 seconds. Data was analyzed using the 1:1 binding model of analysis software (BIAevaluation Software, version 4.1) to calculate an association rate constant kon, a dissociation rate constant koff, and a dissociation constant (K_D; K_D = koff/kon). As a result, the K_D value was 0.41 nM for H-1040, 3.5 nM for H-1143, 3.9 nM for H-1140, 0.40 nM for H-2040, 1.7 nM for H-2143, and 1.8 nM for H-2140.

11)-2 Activation of ROBO4 downstream signal

This test was conducted according to the method of 4)-1 except that: human IgG was used as a negative control; and Human IgG, H-1040, H-1140, H-1143, H-2040, H-2140, H-2143, or cMAb1-2 was added at a final concentration of 0.63 μg/mL to a medium. As a result, the negative control human IgG did not influence the IL-8 promoter activity in the cells transiently expressing human ROBO4, whereas all the anti-ROBO4 humanized antibodies increased the IL-8 promoter activity at a level equivalent to cMAb1-2 (Figure 49). These results demonstrated that all the anti-ROBO4 humanized antibodies activated the downstream signal of ROBO4, as in MAb1.

11)-3 HUVEC migration test

This test was conducted according to the method of 4)-2 except that: human IgG was used as a negative control; human IgG, H-1143, H-2140, or H-2143 was added at a final concentration of 0.5 μg/mL to a medium; and the fluorescence intensity (excitation

wavelength/fluorescent wavelength: 485 nm/538 nm) of each well was measured using a plate reader (EnVision: Perkin Elmer, Inc.). As a result, H-1143, H-2140, or H-2143 suppressed the migration of HUVEC induced by bFGF (Figure 50). Additionally, H-1140 showed the suppressive activity against HUVEC migration. These results demonstrated that H-1140, H-1143, H-2140, or H-2143 suppressed the migration of HUVEC.

11)-4 Cross-species reactivity

11)-4-1 Preparation of antigen gene-expressing cell

The cells were prepared according to the method of 4)-3-1.

11)-4-2 Flow cytometry analysis

This test was conducted according to the method of 4)-3-2 except that: human IgG was used as a negative control; FITC-AffiniPure Goat Anti-Human IgG, Fcγ Fragment Specific (manufactured by Jackson ImmunoResearch Laboratories, Inc.) was used as a secondary antibody; and a flow cytometer (FC500; manufactured by Beckman Coulter, Inc.) was used as a detector. As a result, H-1143, H-2140, or H-2143 did not bind to mouse ROBO4 or rat ROBO4, but was shown to bind to human ROBO4 and cynomolgus monkey ROBO4, as in the parent antibody MAb1 (Figures 51, 52, and 53). Additionally, H-1140 showed the same result as H-1143, H-2140 and H-2143.

11)-5 Binding specificity of H-1140, H-1143, H-2140, or H-2143

11)-5-1 Preparation of antigen gene-expressing cell

The cells were prepared according to the method of 4)-4-1 except that pCI-FLAG-hROBO4-28 was used as a human ROBO4 expression vector.

11)-5-2 Flow cytometry analysis

This test was conducted according to the method of 4)-4-2 except that: human IgG or mouse IgG2A was used as a negative control; Fluorescein-conjugated goat IgG fraction to mouse IgG (manufactured by Cappel Laboratories, Inc.) or FITC-AffiniPure Goat Anti-Human IgG, Fcγ Fragment Specific was used as a secondary antibody; and a flow cytometer (FC500) was used as a detector. As a result, H-1143, H-2140, or H-2143 did not bind to hROBO1, hROBO2, or hROBO3, but was shown to specifically bind to hROBO4, as in the parent antibody MAb1 (Figure 54). Additionally, H-1140 showed the same result as H-1143, H-2140 and H-2143. In this context, hROBO4, hROBO1, hROBO2, and hROBO3 were each confirmed to be expressed on the cell membrane using the positive control antibody (Figure 54).

11)-6 Drug efficacy evaluation in monkey models with laser-induced choroidal neovascularization

11)-6-1 Anesthesia

Medetomidine hydrochloride was intramuscularly injected at a dose of 0.08 mg/kg to each cynomolgus monkey. 15 minutes thereafter, ketamine hydrochloride was intramuscularly injected thereto at a dose of 15 mg/kg.

11)-6-2 Model preparation

Each cynomolgus monkey anesthetized in 11)-6-1 was retained on a stainless operating table in the supine position. 5 mg/mL tropicamide-5 mg/mL phenylephrine hydrochloride mixture eye drops were applied to the eyes for mydriasis. The macular region of the retina was thermally damaged by laser irradiation (quantity of heat irradiated: 500 mW, irradiation time: 0.1 seconds, spot size: 50 μm, the number of spots: 9 spots) using a green laser photocoagulator OcuLight GLx. After the operation, Cravit eye drops were added dropwise to the operated eyes.

11)-6-3 Administration of test substance

At day 7 after the model preparation, each cynomolgus monkey was anesthetized and then retained on a stainless operating table in the supine position. Then, PA IODO Ophthalmic and Eye washing Solution (Nitten Pharmaceutical Co., Ltd.) diluted 4-fold with sterile purified water was applied to the eyes to disinfect the external eyes. A 33G needle was inserted into the vitreous body from the conjunctiva, and saline or 0.05 mL of H-2143 adjusted to 1.1 mg/0.05 mL was injected thereto using a 1-mL syringe. After completion of the administration, Rinderon-A Ointment for Eye and Ear was applied to the conjunctiva using a swab and spread throughout the surfaces of the eyes by the opening and closing of the eyes.

11)-6-4 Drug efficacy evaluation

At days 7, 14, and 21 after the model preparation, the ocular fundus was photographed by a routine method using a hybrid fundus camera CX-1 under anesthesia. Then, fluorescein was intravenously injected thereto at a dose of 0.05 mL/Kg. After completion of the intravenous injection of fluorescein, fluorescent angiography was performed every 1 minute up to 10 minutes later, and image data was stored. From the image data, severity was classified at a scale of grades 1 to 5 based on the fluorescence intensity of each laser-irradiated site at which fluorescein accumulated according to the method of Zahn G et al. (Zahn G et al., Arch. Ophthalmol. 2009 127: 1329-1335). Then, the ratio of a site corresponding to grades 4 and 5 in the severity classification among all the laser-irradiated sites was calculated on the percentage basis to evaluate choroidal neovascularization. As a result of comparing choroidal neovascularization between the saline-administered group and the H-2143-administered group, the ratio of grades 4 and 5 increased in the saline-administered group with a lapse of time from the model preparation, whereas such increase was not observed in the H-2143-administered

group. This means that the administration of H-2143 suppressed laser-induced choroidal neovascularization (Figure 55).

[Industrial Applicability]

Use of an antibody provided by the present invention enables the treatment or prevention of an angiogenic disease such as exudative age-related macular degeneration, diabetic retinopathy, macular edema, benign or malignant tumor, atherosclerosis, retrolental fibroplasia, angioma, chronic inflammation, ocular neovascular disease, proliferative retinopathy, neovascular glaucoma, immune rejection of a corneal tissue transplant or other tissue transplants, rheumatoid arthritis, psoriasis, acute inflammation, sepsis, or obesity, and the examination or diagnosis of the angiogenic disease.

[Free Text of Sequence Listing]

SEQ ID NO: 1: Nucleotide sequence of full-length human ROBO4 cDNA (Figure 13)

SEQ ID NO: 2: Amino acid sequence of human ROBO4 (Figure 14)

SEQ ID NO: 3: Nucleotide sequence encoding the amino acid sequence of N-terminal FLAG-tagged full-length human ROBO4

SEQ ID NO: 4: Amino acid sequence of N-terminal FLAG-tagged full-length human ROBO4

SEQ ID NO: 5: Nucleotide sequence encoding the amino acid sequence of an N-terminal FLAG-tagged extracellular region/domain deletion variant of human ROBO4 consisting of an amino acid sequence of amino acid Nos. 46 to 1007 of SEQ ID NO: 2

SEQ ID NO: 6: Amino acid sequence of an N-terminal FLAG-tagged extracellular region/domain deletion variant of human ROBO4 consisting of an amino acid sequence of amino acid Nos. 46 to 1007 of SEQ ID NO: 2

SEQ ID NO: 7: Nucleotide sequence encoding the amino acid sequence of an N-terminal FLAG-tagged extracellular region/domain deletion variant of human ROBO4 consisting of an amino acid sequence of amino acid Nos. 132 to 1007 of SEQ ID NO: 2

SEQ ID NO: 8: Amino acid sequence of an N-terminal FLAG-tagged extracellular region/domain deletion variant of human ROBO4 consisting of an amino acid sequence of amino acid Nos. 132 to 1007 of SEQ ID NO: 2

SEQ ID NO: 9: Nucleotide sequence encoding the amino acid sequence of an N-terminal FLAG-tagged extracellular region/domain deletion variant of human ROBO4 consisting of an amino acid sequence of amino acid Nos. 210 to 1007 of SEQ ID NO: 2

SEQ ID NO: 10: Amino acid sequence of an N-terminal FLAG-tagged extracellular region/domain deletion variant of human ROBO4 consisting of an amino acid sequence of amino acid Nos. 210 to 1007 of SEQ ID NO: 2

SEQ ID NO: 11: Nucleotide sequence encoding the amino acid sequence of an N-terminal FLAG-tagged extracellular region/domain deletion variant of human ROBO4 consisting of an amino acid sequence of amino acid Nos. 225 to 1007 of SEQ ID NO: 2

SEQ ID NO: 12: Amino acid sequence of an N-terminal FLAG-tagged extracellular region/domain deletion variant of human ROBO4 consisting of an amino acid sequence of amino acid Nos. 225 to 1007 of SEQ ID NO: 2

SEQ ID NO: 13: Nucleotide sequence encoding the amino acid sequence of an N-terminal FLAG-tagged extracellular region/domain deletion variant of human ROBO4 consisting of an amino acid sequence of amino acid Nos. 341 to 1007 of SEQ ID NO: 2

SEQ ID NO: 14: Amino acid sequence of an N-terminal FLAG-tagged extracellular region/domain deletion variant of human ROBO4 consisting of an amino acid sequence of amino acid Nos. 341 to 1007 of SEQ ID NO: 2

SEQ ID NO: 15: Nucleotide sequence of mouse ROBO4 cDNA

SEQ ID NO: 16: Amino acid sequence of mouse ROBO4

SEQ ID NO: 17: Nucleotide sequence of rat ROBO4 cDNA

SEQ ID NO: 18: Amino acid sequence of rat ROBO4

SEQ ID NO: 19: Nucleotide sequence of monkey ROBO4 cDNA1

SEQ ID NO: 20: Monkey ROBO4 amino acid sequence 1

SEQ ID NO: 21: Nucleotide sequence of monkey ROBO4 cDNA2

SEQ ID NO: 22: Monkey ROBO4 amino acid sequence 2

SEQ ID NO: 23: Nucleotide sequence of human ROBO1 cDNA

SEQ ID NO: 24: Amino acid sequence of human ROBO1

SEQ ID NO: 25: Nucleotide sequence of human ROBO2 cDNA

SEQ ID NO: 26: Amino acid sequence of human ROBO2

SEQ ID NO: 27: Nucleotide sequence of human ROBO3 cDNA

SEQ ID NO: 28: Amino acid sequence of human ROBO4

SEQ ID NO: 29: Nucleotide sequence of an IL-8 promoter region

SEQ ID NO: 30: Nucleotide sequence of cDNA encoding MAb1 heavy chain variable region (Figure 15)

SEQ ID NO: 31: Amino acid sequence of MAb1 heavy chain variable region (Figure 16)

SEQ ID NO: 32: Nucleotide sequence of cDNA encoding MAb1 light chain variable region (Figure 17)

SEQ ID NO: 33: Amino acid sequence of MAb1 light chain variable region (Figure 18)

SEQ ID NO: 34: Nucleotide sequence comprising cDNA encoding the amino acids of human κ chain secretory signal and human κ chain constant region

SEQ ID NO: 35: Nucleotide sequence comprising cDNA encoding the amino acids of human heavy chain signal sequence and human IgG1 constant region

SEQ ID NO: 36: Nucleotide sequence comprising cDNA encoding the amino acids of human heavy chain signal sequence and human IgG2 constant region

SEQ ID NO: 37: Nucleotide sequence of cDNA encoding cMAb1 light chain (Figure 19)

SEQ ID NO: 38: Amino acid sequence of cMAb1 light chain (Figure 20)

SEQ ID NO: 39: Nucleotide sequence of cDNA encoding cMAb1-1 heavy chain (Figure 21)

SEQ ID NO: 40: Amino acid sequence of cMAb1-1 heavy chain (Figure 22)

SEQ ID NO: 41: Nucleotide sequence of cDNA encoding cMAb1-2 heavy chain (Figure 23)

SEQ ID NO: 42: Amino acid sequence of cMAb1-2 heavy chain (Figure 24)

SEQ ID NO: 43: Nucleotide sequence encoding the amino acid sequence of MAb1 heavy chain CDRH1

SEQ ID NO: 44: Amino acid sequence of MAb1 heavy chain CDRH1 (Figure 25)

SEQ ID NO: 45: Nucleotide sequence encoding the amino acid sequence of MAb1 heavy chain CDRH2

SEQ ID NO: 46: Amino acid sequence of MAb1 heavy chain CDRH2 (Figure 26)

SEQ ID NO: 47: Nucleotide sequence encoding the amino acid sequence of MAb1 heavy chain CDRH3

SEQ ID NO: 48: Amino acid sequence of MAb1 heavy chain CDRH3 (Figure 27)

SEQ ID NO: 49: Nucleotide sequence encoding the amino acid sequence of MAb1 light chain CDRL1

SEQ ID NO: 50: Amino acid sequence of MAb1 light chain CDRL1 (Figure 28)

SEQ ID NO: 51: Nucleotide sequence encoding the amino acid sequence of MAb1 light chain CDRL2

SEQ ID NO: 52: Amino acid sequence of MAb1 light chain CDRL2 (Figure 29)

SEQ ID NO: 53: Nucleotide sequence encoding the amino acid sequence of MAb1 light chain CDRL3

SEQ ID NO: 54: Amino acid sequence of MAb1 light chain CDRL3 (Figure 30)

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SEQ ID NO: 56: Amino acid sequence of hMAb1-H1-type heavy chain (Figure 32)

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SEQ ID NO: 61: Nucleotide sequence of cDNA encoding hMAb1-H4-type heavy chain (Figure 37)

SEQ ID NO: 62: Amino acid sequence of hMAb1-H4-type heavy chain (Figure 38)

SEQ ID NO: 63: Nucleotide sequence of cDNA encoding hMAb1-L1-type light chain (Figure 39)

SEQ ID NO: 64: Amino acid sequence of hMAb1-L1-type heavy chain (Figure 40)

SEQ ID NO: 65: Nucleotide sequence of cDNA encoding hMAb1-L2-type heavy chain (Figure 41)

SEQ ID NO: 66: Amino acid sequence of hMAb1-L2-type heavy chain (Figure 42)

SEQ ID NO: 67: CDRH1 of hMAb1-H2 or hMAb1-H4-type heavy chain (Figure 43)

SEQ ID NO: 68: CDRH2 of hMAb1-H2 or hMAb1-H4-type heavy chain (Figure 44)

SEQ ID NO: 69: CDRH3 of hMAb1-H2 or hMAb1-H4-type heavy chain (Figure 45)

SEQ ID NO: 70: CDRL1 of hMAb1-L2-type light chain (Figure 46)

SEQ ID NO: 71: CDRL2 of hMAb1-L2-type light chain (Figure 47)

SEQ ID NO: 72: CDRL3 of hMAb1-L2-type light chain (Figure 48)

[Claim 1]

An antibody having properties described in the following (I) to (III) or a functional fragment thereof:

(I) binding to the ROBO4 protein with a K_D value of 1×10^{-8} M or lower;
(II) suppressing or inhibiting vascular endothelial cell migration in the absence of a cross-linking antibody in vitro; and
(III) suppressing or inhibiting angiogenesis in vivo,
wherein the antibody consists of a heavy chain comprising CDRH1 consisting of the amino acid sequence represented by SEQ ID NO: 44 (Figure 25), CDRH2 consisting of the amino acid sequence represented by SEQ ID NO: 46 (Figure 26) or the amino acid sequence represented by SEQ ID NO: 68 (Figure 44), and CDRH3 consisting of the amino acid sequence represented by SEQ ID NO: 48 (Figure 27), and a light chain comprising CDRL1 consisting of the amino acid sequence represented by SEQ ID NO: 50 (Figure 28) or the amino acid sequence represented by SEQ ID NO: 70 (Figure 46), CDRL2 consisting of the amino acid sequence represented by SEQ ID NO: 52 (Figure 29), and CDRL3 consisting of the amino acid sequence represented by SEQ ID NO: 54 (Figure 30).

[Claim 2]

The antibody or the functional fragment thereof according to claim 1, wherein the ROBO4 protein is the human ROBO4 protein.

[Claim 3]

The antibody or the functional fragment thereof according to claim 1, wherein the ROBO4 protein is a protein consisting of an amino acid sequence of amino acid Nos. 1 to 1007 of SEQ ID NO: 2.

[Claim 4]

The antibody or the functional fragment thereof according to claim 1, wherein the ROBO4 protein is a protein consisting of an amino acid sequence of amino acid Nos. 46 to 1007 of SEQ ID NO: 2.

[Claim 5]

The antibody or the functional fragment thereof according to claim 3 or 4, wherein the antibody or the functional fragment thereof binds to a site consisting of an amino acid sequence of amino acid Nos. 132 to 209 of SEQ ID NO: 2.

[Claim 6]

The antibody or the functional fragment thereof according to claim 1, wherein the antibody or the functional fragment thereof is a monoclonal antibody or a functional fragment thereof.

[Claim 7]

The antibody or the functional fragment thereof according to any one of claims 1 to 6, wherein the antibody comprises a heavy chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 31 (Figure 16), and a light chain variable region consisting of the amino acid sequence represented by SEQ ID NO: 33 (Figure 18).

[Claim 8]

The antibody or the functional fragment thereof according to any one of claims 1 to 6, wherein the antibody comprises any one heavy chain variable region selected from the following a) to d) and a light chain variable region selected from e) and f):

a) a heavy chain (hMAb1-H1-type) variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 56 (Figure 32),
b) a heavy chain (hMAb1-H2-type) variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 58 (Figure 34),
c) a heavy chain (hMAb1-H3-type) variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 60 (Figure 36), and
d) a heavy chain (hMAb1-H4-type) variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 62 (Figure 38); and
e) a light chain (hMAb1-L1-type) variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40), and
f) a light chain (hMAb1-L2-type) variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 66 (Figure 42).

[Claim 9]

The antibody or the functional fragment thereof according to any one of claims 1 to 6, wherein the antibody comprises any one of the following combinations 1) to 6) of a heavy chain variable region and a light chain variable region:

1) a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 58 (Figure 34) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40),

2) a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 58 (Figure 34) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 66 (Figure 42),

3) a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 62 (Figure 38) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 66 (Figure 42),

4) a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 62 (Figure 38) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40),

5) a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 56 (Figure 32) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40), and

6) a heavy chain variable region consisting of an amino acid sequence represented by amino acid Nos. 20 to 137 of SEQ ID NO: 60 (Figure 36) and a light chain variable region consisting of an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 64 (Figure 40).

[Claim 10]

The antibody or the functional fragment thereof according to any one of claims 1 to 9, wherein the antibody or the functional fragment thereof is a chimeric antibody or a functional fragment thereof.

[Claim 11]

The antibody or the functional fragment thereof according to any one of claims 1 to 9, wherein the antibody or the functional fragment thereof is a humanized antibody or a functional fragment thereof.

[Claim 12]

The antibody or the functional fragment thereof according to any one of claims 1 to 11, wherein the antibody comprises a human IgG1 or human IgG2 heavy chain constant region.

[Claim 13]

The antibody or the functional fragment thereof according to any one of claims 1 to 6, wherein the antibody or the functional fragment thereof binds to a site on an antigen recognized by the antibody according to claims 8 or 9.

[Claim 14]

The antibody or the functional fragment thereof according to any one of claims 1 to 6, wherein the antibody or the functional fragment thereof competes with any one antibody according to claims 8 or 9 for the binding to ROBO4 protein.

[Claim 15]

The antibody according to any one of claims 1 to 6, wherein the antibody comprises any one of the following combinations 1) to 6) of a heavy chain and a light chain:

1) a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 58 (Figure 34) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 64 (Figure 40) (H-1140),

2) a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 58 (Figure 34) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 66 (Figure 42) (H-1143),

3) a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 62 (Figure 38) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 66 (Figure 42) (H-2143).

4) a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 62 (Figure 38) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 64 (Figure 40) (H-2140),

5) a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 56 (Figure 32) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 64 (Figure 40) (H-1040), and

6) a heavy chain consisting of an amino acid sequence represented by amino acid Nos. 20 to 463 of SEQ ID NO: 60 (Figure 36) and a light chain consisting of an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 64 (Figure 40) (H-2040).

[Claim 16]

An antibody comprising a light chain and modified form of a heavy chain as defined in claim 15, wherein said modified form lacks one to ten carboxyl-terminal amino acid(s) of said heavy chain.

[Claim 17]

The antibody according to any one of claims 1 to 6, wherein the antibody is 95% or more identical in the amino acid sequence to any one antibody according to claim 15; has a K_D value of 1×10^{-8} M or lower for a human ROBO4; suppresses or inhibits vascular endothelial cell migration in the absence of a cross-linking antibody in vitro; and suppresses or inhibits angiogenesis in vivo.

[Claim 18]

The antibody or the functional fragment thereof according to claim 13, wherein the antibody or the functional fragment thereof is a human antibody or a functional fragment thereof.

[Claim 19]

A nucleotide sequence selected from the group consisting of the following (I) to (III):

(I) a nucleotide sequence comprising a nucleotide sequence encoding the partial or whole amino acid sequence of the heavy chain or light chain of an antibody according to any one of claims 1 to 18,

(II) a nucleotide sequence consisting of a nucleotide sequence comprising a nucleotide sequence encoding the partial or whole amino acid sequence of the heavy chain or light chain of an antibody according to any one of claims 1 to 18, and

(III) a nucleotide sequence consisting of a nucleotide sequence encoding the partial or whole amino acid sequence of the heavy chain or light chain of an antibody according to any one of claims 1 to 18.

[Claim 20]

A recombinant vector containing an insert of a nucleotide sequence according to claim 19.

[Claim 21]

A pharmaceutical composition comprising an antibody or a functional fragment thereof according to any one of claims 1 to 18 as an active ingredient.

[Claim 22]

The pharmaceutical composition according to claim 21, wherein the pharmaceutical composition is for use in treating or preventing an angiogenic disease.

[Claim 23]

The pharmaceutical composition for the use according to claim 22, wherein the angiogenic disease is exudative age-related macular degeneration, diabetic retinopathy, macular edema, benign or malignant tumor, atherosclerosis, retrolental fibroplasia, angioma, chronic inflammation, ocular neovascular disease, proliferative retinopathy, neovascular glaucoma, immune rejection of a corneal tissue transplant or other tissue transplants, rheumatoid arthritis, psoriasis, acute inflammation, sepsis, or obesity.

[Claim 24]

The pharmaceutical composition for the use according to claim 22, wherein the angiogenic disease is exudative age-related macular degeneration, diabetic retinopathy, macular

edema, retrolental fibroplasia, ocular neovascular disease, proliferative retinopathy, neovascular glaucoma, or immune rejection of a corneal tissue transplant.

[Claim 25]

An angiogenesis inhibitor comprising an antibody or a functional fragment thereof according to any one of claims 1 to 18 as an active ingredient.

[Claim 26]

The pharmaceutical composition according to any one of claims 21 to 24, wherein said composition is to be used in combination with a further therapeutic or prophylactic agent.

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<211> 1015
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<213> Artificial Sequence

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<400> 4

Met Gly Ser Gly Gly Asp Ser Leu Leu Gly Gly Arg Gly Ser Leu Pro
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Leu Leu Leu Leu Leu Ile Met Gly Gly Met Ala Asp Tyr Lys Asp Asp
20 25 30

Asp Asp Lys Gln Asp Ser Pro Pro Gln Ile Leu Val His Pro Gln Asp
35 40 45

Gln Leu Phe Gln Gly Pro Gly Pro Ala Arg Met Ser Cys Gln Ala Ser
50 55 60

Gly Gln Pro Pro Pro Thr Ile Arg Trp Leu Leu Asn Gly Gln Pro Leu
65 70 75 80

Ser Met Val Pro Pro Asp Pro His His Leu Leu Pro Asp Gly Thr Leu
85 90 95

Leu Leu Leu Gln Pro Pro Ala Arg Gly His Ala His Asp Gly Gln Ala
100 105 110

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Leu Ser Thr Asp Leu Gly Val Tyr Thr Cys Glu Ala Ser Asn Arg Leu
        115                 120                 125

Gly Thr Ala Val Ser Arg Gly Ala Arg Leu Ser Val Ala Val Leu Arg
    130                 135                 140

Glu Asp Phe Gln Ile Gln Pro Arg Asp Met Val Ala Val Val Gly Glu
145                 150                 155                 160

Gln Phe Thr Leu Glu Cys Gly Pro Pro Trp Gly His Pro Glu Pro Thr
                165                 170                 175

Val Ser Trp Trp Lys Asp Gly Lys Pro Leu Ala Leu Gln Pro Gly Arg
            180                 185                 190

His Thr Val Ser Gly Gly Ser Leu Leu Met Ala Arg Ala Glu Lys Ser
        195                 200                 205

Asp Glu Gly Thr Tyr Met Cys Val Ala Thr Asn Ser Ala Gly His Arg
    210                 215                 220

Glu Ser Arg Ala Ala Arg Val Ser Ile Gln Glu Pro Gln Asp Tyr Thr
225                 230                 235                 240

Glu Pro Val Glu Leu Leu Ala Val Arg Ile Gln Leu Glu Asn Val Thr
                245                 250                 255

Leu Leu Asn Pro Asp Pro Ala Glu Gly Pro Lys Pro Arg Pro Ala Val
            260                 265                 270

Trp Leu Ser Trp Lys Val Ser Gly Pro Ala Ala Pro Ala Gln Ser Tyr
        275                 280                 285

Thr Ala Leu Phe Arg Thr Gln Thr Ala Pro Gly Gly Gln Gly Ala Pro
    290                 295                 300

Trp Ala Glu Glu Leu Leu Ala Gly Trp Gln Ser Ala Glu Leu Gly Gly
305                 310                 315                 320

Leu His Trp Gly Gln Asp Tyr Glu Phe Lys Val Arg Pro Ser Ser Gly
                325                 330                 335

Arg Ala Arg Gly Pro Asp Ser Asn Val Leu Leu Leu Arg Leu Pro Glu
            340                 345                 350

Lys Val Pro Ser Ala Pro Pro Gln Glu Val Thr Leu Lys Pro Gly Asn
        355                 360                 365

Gly Thr Val Phe Val Ser Trp Val Pro Pro Pro Ala Glu Asn His Asn
    370                 375                 380
```

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Gly Ile Ile Arg Gly Tyr Gln Val Trp Ser Leu Gly Asn Thr Ser Leu
385                 390                 395                 400

Pro Pro Ala Asn Trp Thr Val Val Gly Glu Gln Thr Gln Leu Glu Ile
                405                 410                 415

Ala Thr His Met Pro Gly Ser Tyr Cys Val Gln Val Ala Ala Val Thr
            420                 425                 430

Gly Ala Gly Ala Gly Glu Pro Ser Arg Pro Val Cys Leu Leu Leu Glu
        435                 440                 445

Gln Ala Met Glu Arg Ala Thr Gln Glu Pro Ser Glu His Gly Pro Trp
    450                 455                 460

Thr Leu Glu Gln Leu Arg Ala Thr Leu Lys Arg Pro Glu Val Ile Ala
465                 470                 475                 480

Thr Cys Gly Val Ala Leu Trp Leu Leu Leu Leu Gly Thr Ala Val Cys
                485                 490                 495

Ile His Arg Arg Arg Arg Ala Arg Val His Leu Gly Pro Gly Leu Tyr
            500                 505                 510

Arg Tyr Thr Ser Glu Asp Ala Ile Leu Lys His Arg Met Asp His Ser
        515                 520                 525

Asp Ser Gln Trp Leu Ala Asp Thr Trp Arg Ser Thr Ser Gly Ser Arg
    530                 535                 540

Asp Leu Ser Ser Ser Ser Ser Leu Ser Ser Arg Leu Gly Ala Asp Ala
545                 550                 555                 560

Arg Asp Pro Leu Asp Cys Arg Arg Ser Leu Leu Ser Trp Asp Ser Arg
                565                 570                 575

Ser Pro Gly Val Pro Leu Leu Pro Asp Thr Ser Thr Phe Tyr Gly Ser
            580                 585                 590

Leu Ile Ala Glu Leu Pro Ser Ser Thr Pro Ala Arg Pro Ser Pro Gln
        595                 600                 605

Val Pro Ala Val Arg Arg Leu Pro Pro Gln Leu Ala Gln Leu Ser Ser
    610                 615                 620

Pro Cys Ser Ser Ser Asp Ser Leu Cys Ser Arg Arg Gly Leu Ser Ser
625                 630                 635                 640

Pro Arg Leu Ser Leu Ala Pro Ala Glu Ala Trp Lys Ala Lys Lys Lys
                645                 650                 655
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Gln Glu Leu Gln His Ala Asn Ser Ser Pro Leu Leu Arg Gly Ser His
            660                 665                 670

Ser Leu Glu Leu Arg Ala Cys Glu Leu Gly Asn Arg Gly Ser Lys Asn
        675                 680                 685

Leu Ser Gln Ser Pro Gly Ala Val Pro Gln Ala Leu Val Ala Trp Arg
    690                 695                 700

Ala Leu Gly Pro Lys Leu Leu Ser Ser Ser Asn Glu Leu Val Thr Arg
705                 710                 715                 720

His Leu Pro Pro Ala Pro Leu Phe Pro His Glu Thr Pro Pro Thr Gln
                725                 730                 735

Ser Gln Gln Thr Gln Pro Pro Val Ala Pro Gln Ala Pro Ser Ser Ile
            740                 745                 750

Leu Leu Pro Ala Ala Pro Ile Pro Ile Leu Ser Pro Cys Ser Pro Pro
        755                 760                 765

Ser Pro Gln Ala Ser Ser Leu Ser Gly Pro Ser Pro Ala Ser Ser Arg
    770                 775                 780

Leu Ser Ser Ser Ser Leu Ser Ser Leu Gly Glu Asp Gln Asp Ser Val
785                 790                 795                 800

Leu Thr Pro Glu Glu Val Ala Leu Cys Leu Glu Leu Ser Glu Gly Glu
                805                 810                 815

Glu Thr Pro Arg Asn Ser Val Ser Pro Met Pro Arg Ala Pro Ser Pro
            820                 825                 830

Pro Thr Thr Tyr Gly Tyr Ile Ser Val Pro Thr Ala Ser Glu Phe Thr
        835                 840                 845

Asp Met Gly Arg Thr Gly Gly Gly Val Gly Pro Lys Gly Gly Val Leu
    850                 855                 860

Leu Cys Pro Pro Arg Pro Cys Leu Thr Pro Thr Pro Ser Glu Gly Ser
865                 870                 875                 880

Leu Ala Asn Gly Trp Gly Ser Ala Ser Glu Asp Asn Ala Ala Ser Ala
                885                 890                 895

Arg Ala Ser Leu Val Ser Ser Ser Asp Gly Ser Phe Leu Ala Asp Ala
            900                 905                 910

His Phe Ala Arg Ala Leu Ala Val Ala Val Asp Ser Phe Gly Phe Gly
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915 920 925

Leu Glu Pro Arg Glu Ala Asp Cys Val Phe Ile Asp Ala Ser Ser Pro
930 935 940

Pro Ser Pro Arg Asp Glu Ile Phe Leu Thr Pro Asn Leu Ser Leu Pro
945 950 955 960

Leu Trp Glu Trp Arg Pro Asp Trp Leu Glu Asp Met Glu Val Ser His
965 970 975

Thr Gln Arg Leu Gly Arg Gly Met Pro Pro Trp Pro Pro Asp Ser Gln
980 985 990

Ile Ser Ser Gln Arg Ser Gln Leu His Cys Arg Met Pro Lys Ala Gly
995 1000 1005

Ala Ser Pro Val Asp Tyr Ser
1010 1015

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<211> 2997
<212> DNA
<213> Artificial Sequence

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atgagctgcc aagcctcagg ccagccacct cccaccatcc gctggttgct gaatgggcag 180

cccctgagca tggtgccccc agacccacac cacctcctgc ctgatgggac ccttctgctg 240

ctacagcccc ctgcccgggg acatgcccac gatggccagg ccctgtccac agacctgggt 300

gtctacacat gtgaggccag caaccggctt ggcacggcag tcagcagagg cgctcggctg 360

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ggtgagcagt ttactctgga atgtgggccg ccctggggcc acccagagcc cacagtctca 480

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<211> 999
<212> PRT
<213> Artificial Sequence

<220>
<223> FLAG-hROBO4-46aa

<400> 6

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Leu Leu Leu Leu Leu Ile Met Gly Gly Met Ala Asp Tyr Lys Asp Asp
20 25 30

Asp Asp Lys Pro Gly Pro Ala Arg Met Ser Cys Gln Ala Ser Gly Gln
35 40 45

Pro Pro Pro Thr Ile Arg Trp Leu Leu Asn Gly Gln Pro Leu Ser Met
50 55 60

Val Pro Pro Asp Pro His His Leu Leu Pro Asp Gly Thr Leu Leu Leu
65 70 75 80

Leu Gln Pro Pro Ala Arg Gly His Ala His Asp Gly Gln Ala Leu Ser
85 90 95

Thr Asp Leu Gly Val Tyr Thr Cys Glu Ala Ser Asn Arg Leu Gly Thr
100 105 110

Ala Val Ser Arg Gly Ala Arg Leu Ser Val Ala Val Leu Arg Glu Asp
115 120 125

Phe Gln Ile Gln Pro Arg Asp Met Val Ala Val Val Gly Glu Gln Phe
130 135 140

Thr Leu Glu Cys Gly Pro Pro Trp Gly His Pro Glu Pro Thr Val Ser
145 150 155 160

Trp Trp Lys Asp Gly Lys Pro Leu Ala Leu Gln Pro Gly Arg His Thr
165 170 175

Val Ser Gly Gly Ser Leu Leu Met Ala Arg Ala Glu Lys Ser Asp Glu
180 185 190

Gly Thr Tyr Met Cys Val Ala Thr Asn Ser Ala Gly His Arg Glu Ser
195 200 205

Arg Ala Ala Arg Val Ser Ile Gln Glu Pro Gln Asp Tyr Thr Glu Pro
210 215 220

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Val Glu Leu Leu Ala Val Arg Ile Gln Leu Glu Asn Val Thr Leu Leu
225                 230                 235                 240

Asn Pro Asp Pro Ala Glu Gly Pro Lys Pro Arg Pro Ala Val Trp Leu
                245                 250                 255

Ser Trp Lys Val Ser Gly Pro Ala Ala Pro Ala Gln Ser Tyr Thr Ala
            260                 265                 270

Leu Phe Arg Thr Gln Thr Ala Pro Gly Gly Gln Gly Ala Pro Trp Ala
        275                 280                 285

Glu Glu Leu Leu Ala Gly Trp Gln Ser Ala Glu Leu Gly Gly Leu His
    290                 295                 300

Trp Gly Gln Asp Tyr Glu Phe Lys Val Arg Pro Ser Ser Gly Arg Ala
305                 310                 315                 320

Arg Gly Pro Asp Ser Asn Val Leu Leu Leu Arg Leu Pro Glu Lys Val
                325                 330                 335

Pro Ser Ala Pro Pro Gln Glu Val Thr Leu Lys Pro Gly Asn Gly Thr
            340                 345                 350

Val Phe Val Ser Trp Val Pro Pro Pro Ala Glu Asn His Asn Gly Ile
        355                 360                 365

Ile Arg Gly Tyr Gln Val Trp Ser Leu Gly Asn Thr Ser Leu Pro Pro
    370                 375                 380

Ala Asn Trp Thr Val Val Gly Glu Gln Thr Gln Leu Glu Ile Ala Thr
385                 390                 395                 400

His Met Pro Gly Ser Tyr Cys Val Gln Val Ala Ala Val Thr Gly Ala
                405                 410                 415

Gly Ala Gly Glu Pro Ser Arg Pro Val Cys Leu Leu Leu Glu Gln Ala
            420                 425                 430

Met Glu Arg Ala Thr Gln Glu Pro Ser Glu His Gly Pro Trp Thr Leu
        435                 440                 445

Glu Gln Leu Arg Ala Thr Leu Lys Arg Pro Glu Val Ile Ala Thr Cys
    450                 455                 460

Gly Val Ala Leu Trp Leu Leu Leu Leu Gly Thr Ala Val Cys Ile His
465                 470                 475                 480

Arg Arg Arg Arg Ala Arg Val His Leu Gly Pro Gly Leu Tyr Arg Tyr
```

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                485                 490                 495

Thr Ser Glu Asp Ala Ile Leu Lys His Arg Met Asp His Ser Asp Ser
            500                 505                 510

Gln Trp Leu Ala Asp Thr Trp Arg Ser Thr Ser Gly Ser Arg Asp Leu
        515                 520                 525

Ser Ser Ser Ser Ser Leu Ser Ser Arg Leu Gly Ala Asp Ala Arg Asp
    530                 535                 540

Pro Leu Asp Cys Arg Arg Ser Leu Leu Ser Trp Asp Ser Arg Ser Pro
545                 550                 555                 560

Gly Val Pro Leu Leu Pro Asp Thr Ser Thr Phe Tyr Gly Ser Leu Ile
                565                 570                 575

Ala Glu Leu Pro Ser Ser Thr Pro Ala Arg Pro Ser Pro Gln Val Pro
            580                 585                 590

Ala Val Arg Arg Leu Pro Pro Gln Leu Ala Gln Leu Ser Ser Pro Cys
        595                 600                 605

Ser Ser Ser Asp Ser Leu Cys Ser Arg Arg Gly Leu Ser Ser Pro Arg
    610                 615                 620

Leu Ser Leu Ala Pro Ala Glu Ala Trp Lys Ala Lys Lys Lys Gln Glu
625                 630                 635                 640

Leu Gln His Ala Asn Ser Ser Pro Leu Leu Arg Gly Ser His Ser Leu
                645                 650                 655

Glu Leu Arg Ala Cys Glu Leu Gly Asn Arg Gly Ser Lys Asn Leu Ser
            660                 665                 670

Gln Ser Pro Gly Ala Val Pro Gln Ala Leu Val Ala Trp Arg Ala Leu
        675                 680                 685

Gly Pro Lys Leu Leu Ser Ser Ser Asn Glu Leu Val Thr Arg His Leu
    690                 695                 700

Pro Pro Ala Pro Leu Phe Pro His Glu Thr Pro Pro Thr Gln Ser Gln
705                 710                 715                 720

Gln Thr Gln Pro Pro Val Ala Pro Gln Ala Pro Ser Ser Ile Leu Leu
                725                 730                 735

Pro Ala Ala Pro Ile Pro Ile Leu Ser Pro Cys Ser Pro Pro Ser Pro
            740                 745                 750
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Gln Ala Ser Ser Leu Ser Gly Pro Ser Pro Ala Ser Ser Arg Leu Ser
        755                 760                 765

Ser Ser Ser Leu Ser Ser Leu Gly Glu Asp Gln Asp Ser Val Leu Thr
    770                 775                 780

Pro Glu Glu Val Ala Leu Cys Leu Glu Leu Ser Glu Gly Glu Glu Thr
785                 790                 795                 800

Pro Arg Asn Ser Val Ser Pro Met Pro Arg Ala Pro Ser Pro Pro Thr
                805                 810                 815

Thr Tyr Gly Tyr Ile Ser Val Pro Thr Ala Ser Glu Phe Thr Asp Met
            820                 825                 830

Gly Arg Thr Gly Gly Gly Val Gly Pro Lys Gly Gly Val Leu Leu Cys
        835                 840                 845

Pro Pro Arg Pro Cys Leu Thr Pro Thr Pro Ser Glu Gly Ser Leu Ala
    850                 855                 860

Asn Gly Trp Gly Ser Ala Ser Glu Asp Asn Ala Ala Ser Ala Arg Ala
865                 870                 875                 880

Ser Leu Val Ser Ser Ser Asp Gly Ser Phe Leu Ala Asp Ala His Phe
                885                 890                 895

Ala Arg Ala Leu Ala Val Ala Val Asp Ser Phe Gly Phe Gly Leu Glu
            900                 905                 910

Pro Arg Glu Ala Asp Cys Val Phe Ile Asp Ala Ser Ser Pro Pro Ser
        915                 920                 925

Pro Arg Asp Glu Ile Phe Leu Thr Pro Asn Leu Ser Leu Pro Leu Trp
    930                 935                 940

Glu Trp Arg Pro Asp Trp Leu Glu Asp Met Glu Val Ser His Thr Gln
945                 950                 955                 960

Arg Leu Gly Arg Gly Met Pro Pro Trp Pro Pro Asp Ser Gln Ile Ser
                965                 970                 975

Ser Gln Arg Ser Gln Leu His Cys Arg Met Pro Lys Ala Gly Ala Ser
            980                 985                 990

Pro Val Asp Tyr Ser Ala Ser
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<211> 2733
<212> DNA

<213> Artificial Sequence

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<223> FLAG-hROBO4-132na

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gatggctcct tcctcgctga tgctcacttt gcccgggccc tggcagtggc tgtggatagc 2460

tttggtttcg gtctagagcc cagggaggca gactgcgtct tcatagatgc ctcatcacct 2520

ccctccccac gggatgagat cttcctgacc cccaacctct ccctgcccct gtgggagtgg 2580

aggccagact ggttggaaga catggaggtc agccacaccc agcggctggg aaggggggatg 2640

cctccctggc cccctgactc tcagatctct tcccagagaa gtcagctcca ctgtcgtatg 2700

cccaaggctg gtgcttctcc tgtagattac tcc 2733

<210> 8
<211> 911
<212> PRT
<213> Artificial Sequence

<220>
<223> FLAG-hROBO4-132aa

<400> 8

Met Gly Ser Gly Gly Asp Ser Leu Leu Gly Gly Arg Gly Ser Leu Pro
1 5 10 15

Leu Leu Leu Leu Leu Ile Met Gly Gly Met Ala Asp Tyr Lys Asp Asp
20 25 30

Asp Asp Lys Val Ala Val Leu Arg Glu Asp Phe Gln Ile Gln Pro Arg
35 40 45

Asp Met Val Ala Val Val Gly Glu Gln Phe Thr Leu Glu Cys Gly Pro
50 55 60

Pro Trp Gly His Pro Glu Pro Thr Val Ser Trp Trp Lys Asp Gly Lys
65 70 75 80

Pro Leu Ala Leu Gln Pro Gly Arg His Thr Val Ser Gly Gly Ser Leu
85 90 95

Leu Met Ala Arg Ala Glu Lys Ser Asp Glu Gly Thr Tyr Met Cys Val

```
            100                 105                 110

Ala Thr Asn Ser Ala Gly His Arg Glu Ser Arg Ala Ala Arg Val Ser
        115                 120                 125

Ile Gln Glu Pro Gln Asp Tyr Thr Glu Pro Val Glu Leu Leu Ala Val
    130                 135                 140

Arg Ile Gln Leu Glu Asn Val Thr Leu Leu Asn Pro Asp Pro Ala Glu
145                 150                 155                 160

Gly Pro Lys Pro Arg Pro Ala Val Trp Leu Ser Trp Lys Val Ser Gly
                165                 170                 175

Pro Ala Ala Pro Ala Gln Ser Tyr Thr Ala Leu Phe Arg Thr Gln Thr
            180                 185                 190

Ala Pro Gly Gly Gln Gly Ala Pro Trp Ala Glu Glu Leu Leu Ala Gly
        195                 200                 205

Trp Gln Ser Ala Glu Leu Gly Gly Leu His Trp Gly Gln Asp Tyr Glu
    210                 215                 220

Phe Lys Val Arg Pro Ser Ser Gly Arg Ala Arg Gly Pro Asp Ser Asn
225                 230                 235                 240

Val Leu Leu Leu Arg Leu Pro Glu Lys Val Pro Ser Ala Pro Pro Gln
                245                 250                 255

Glu Val Thr Leu Lys Pro Gly Asn Gly Thr Val Phe Val Ser Trp Val
            260                 265                 270

Pro Pro Pro Ala Glu Asn His Asn Gly Ile Ile Arg Gly Tyr Gln Val
        275                 280                 285

Trp Ser Leu Gly Asn Thr Ser Leu Pro Pro Ala Asn Trp Thr Val Val
    290                 295                 300

Gly Glu Gln Thr Gln Leu Glu Ile Ala Thr His Met Pro Gly Ser Tyr
305                 310                 315                 320

Cys Val Gln Val Ala Ala Val Thr Gly Ala Gly Ala Gly Glu Pro Ser
                325                 330                 335

Arg Pro Val Cys Leu Leu Leu Glu Gln Ala Met Glu Arg Ala Thr Gln
            340                 345                 350

Glu Pro Ser Glu His Gly Pro Trp Thr Leu Glu Gln Leu Arg Ala Thr
        355                 360                 365
```

```
Leu Lys Arg Pro Glu Val Ile Ala Thr Cys Gly Val Ala Leu Trp Leu
    370                 375                 380

Leu Leu Leu Gly Thr Ala Val Cys Ile His Arg Arg Arg Arg Ala Arg
385                 390                 395                 400

Val His Leu Gly Pro Gly Leu Tyr Arg Tyr Thr Ser Glu Asp Ala Ile
                405                 410                 415

Leu Lys His Arg Met Asp His Ser Asp Ser Gln Trp Leu Ala Asp Thr
            420                 425                 430

Trp Arg Ser Thr Ser Gly Ser Arg Asp Leu Ser Ser Ser Ser Ser Leu
        435                 440                 445

Ser Ser Arg Leu Gly Ala Asp Ala Arg Asp Pro Leu Asp Cys Arg Arg
    450                 455                 460

Ser Leu Leu Ser Trp Asp Ser Arg Ser Pro Gly Val Pro Leu Leu Pro
465                 470                 475                 480

Asp Thr Ser Thr Phe Tyr Gly Ser Leu Ile Ala Glu Leu Pro Ser Ser
                485                 490                 495

Thr Pro Ala Arg Pro Ser Pro Gln Val Pro Ala Val Arg Arg Leu Pro
            500                 505                 510

Pro Gln Leu Ala Gln Leu Ser Ser Pro Cys Ser Ser Ser Asp Ser Leu
        515                 520                 525

Cys Ser Arg Arg Gly Leu Ser Ser Pro Arg Leu Ser Leu Ala Pro Ala
    530                 535                 540

Glu Ala Trp Lys Ala Lys Lys Lys Gln Glu Leu Gln His Ala Asn Ser
545                 550                 555                 560

Ser Pro Leu Leu Arg Gly Ser His Ser Leu Glu Leu Arg Ala Cys Glu
                565                 570                 575

Leu Gly Asn Arg Gly Ser Lys Asn Leu Ser Gln Ser Pro Gly Ala Val
            580                 585                 590

Pro Gln Ala Leu Val Ala Trp Arg Ala Leu Gly Pro Lys Leu Leu Ser
        595                 600                 605

Ser Ser Asn Glu Leu Val Thr Arg His Leu Pro Pro Ala Pro Leu Phe
    610                 615                 620

Pro His Glu Thr Pro Pro Thr Gln Ser Gln Gln Thr Gln Pro Pro Val
625                 630                 635                 640
```

```
Ala Pro Gln Ala Pro Ser Ser Ile Leu Leu Pro Ala Ala Pro Ile Pro
                645                 650                 655

Ile Leu Ser Pro Cys Ser Pro Pro Ser Pro Gln Ala Ser Ser Leu Ser
            660                 665                 670

Gly Pro Ser Pro Ala Ser Ser Arg Leu Ser Ser Ser Ser Leu Ser Ser
        675                 680                 685

Leu Gly Glu Asp Gln Asp Ser Val Leu Thr Pro Glu Glu Val Ala Leu
    690                 695                 700

Cys Leu Glu Leu Ser Glu Gly Glu Glu Thr Pro Arg Asn Ser Val Ser
705                 710                 715                 720

Pro Met Pro Arg Ala Pro Ser Pro Pro Thr Thr Tyr Gly Tyr Ile Ser
                725                 730                 735

Val Pro Thr Ala Ser Glu Phe Thr Asp Met Gly Arg Thr Gly Gly Gly
            740                 745                 750

Val Gly Pro Lys Gly Gly Val Leu Leu Cys Pro Pro Arg Pro Cys Leu
        755                 760                 765

Thr Pro Thr Pro Ser Glu Gly Ser Leu Ala Asn Gly Trp Gly Ser Ala
    770                 775                 780

Ser Glu Asp Asn Ala Ala Ser Ala Arg Ala Ser Leu Val Ser Ser Ser
785                 790                 795                 800

Asp Gly Ser Phe Leu Ala Asp Ala His Phe Ala Arg Ala Leu Ala Val
                805                 810                 815

Ala Val Asp Ser Phe Gly Phe Gly Leu Glu Pro Arg Glu Ala Asp Cys
            820                 825                 830

Val Phe Ile Asp Ala Ser Ser Pro Pro Ser Pro Arg Asp Glu Ile Phe
        835                 840                 845

Leu Thr Pro Asn Leu Ser Leu Pro Leu Trp Glu Trp Arg Pro Asp Trp
    850                 855                 860

Leu Glu Asp Met Glu Val Ser His Thr Gln Arg Leu Gly Arg Gly Met
865                 870                 875                 880

Pro Pro Trp Pro Pro Asp Ser Gln Ile Ser Ser Gln Arg Ser Gln Leu
                885                 890                 895

His Cys Arg Met Pro Lys Ala Gly Ala Ser Pro Val Asp Tyr Ser
            900                 905                 910
```

<210> 9
<211> 2505
<212> DNA
<213> Artificial Sequence

<220>
<223> FLAG-hROBO4-210na

<400> 9
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ctcatcatgg gaggcatggc tgattacaag gatgacgacg ataagaccaa cagcgcagga 120

catagggaga gccgcgcagc ccgggtttcc atccaggagc cccaggacta cacggagcct 180

gtggagcttc tggctgtgcg aattcagctg gaaaatgtga cactgctgaa cccggatcct 240

gcagagggcc ccaagcctag accggcggtg tggctcagct ggaaggtcag tggccctgct 300

gcgcctgccc aatcttacac ggccttgttc aggacccaga ctgccccggg aggccaggga 360

gctccgtggg cagaggagct gctggccggc tggcagagcg cagagcttgg aggcctccac 420

tggggccaag actacgagtt caaagtgaga ccatcctctg gccgggctcg aggccctgac 480

agcaacgtgc tgctcctgag gctgccggaa aaagtgccca gtgccccacc tcaggaagtg 540

actctaaagc ctggcaatgg cactgtcttt gtgagctggg tcccaccacc tgctgaaaac 600

cacaatggca tcatccgtgg ctaccaggtc tggagcctgg gcaacacatc actgccacca 660

gccaactgga ctgtagttgg tgagcagacc cagctggaaa tcgccaccca tatgccaggc 720

tcctactgcg tgcaagtggc tgcagtcact ggtgctggag ctggggagcc cagtagacct 780

gtctgcctcc ttttagagca ggccatggag cgagccaccc aagaacccag tgagcatggt 840

ccctggaccc tggagcagct gagggctacc ttgaagcggc ctgaggtcat tgccacctgc 900

ggtgttgcac tctggctgct gcttctgggc accgccgtgt gtatccaccg ccggcgccga 960

gctagggtgc acctgggccc aggtctgtac agatatacca gtgaggatgc catcctaaaa 1020

cacaggatgg atcacagtga ctcccagtgg ttggcagaca cttggcgttc cacctctggc 1080

tctcgggacc tgagcagcag cagcagcctc agcagtcggc tgggggcgga tgcccgggac 1140

ccactagact gtcgtcgctc cttgctctcc tgggactccc gaagccccgg cgtgcccctg 1200

cttccagaca ccagcacttt ttatggctcc ctcatcgctg agctgccctc cagtacccca 1260

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ttgtctctgg cccctgcaga ggcttggaag gccaaaaaga agcaggagct gcagcatgcc 1440

aacagttccc cactgctccg gggcagccac tccttggagc tccgggcctg tgagttagga 1500

aatagaggtt ccaagaacct ttcccaaagc ccaggagctg tgccccaagc tctggttgcc 1560

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agtcgcctgt ccagctcctc actgtcatcc ctgggggagg atcaagacag cgtgctgacc 1860

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acagcctcag agttcacgga catgggcagg actggaggag gggtggggcc caaggggga 2040

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aatggttggg gctcagcctc tgaggacaat gccgccagcg ccagagccag ccttgtcagc 2160

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cgtatgccca aggctggtgc ttctcctgta gattactccg ctagc 2505

<210> 10
<211> 835
<212> PRT
<213> Artificial Sequence

<220>
<223> FLAG-hROBO4-210aa

<400> 10

Met Gly Ser Gly Gly Asp Ser Leu Leu Gly Gly Arg Gly Ser Leu Pro
1 5 10 15

Leu Leu Leu Leu Leu Ile Met Gly Gly Met Ala Asp Tyr Lys Asp Asp
20 25 30

Asp Asp Lys Thr Asn Ser Ala Gly His Arg Glu Ser Arg Ala Ala Arg
35 40 45

Val Ser Ile Gln Glu Pro Gln Asp Tyr Thr Glu Pro Val Glu Leu Leu
50 55 60

Ala Val Arg Ile Gln Leu Glu Asn Val Thr Leu Leu Asn Pro Asp Pro
65 70 75 80

Ala Glu Gly Pro Lys Pro Arg Pro Ala Val Trp Leu Ser Trp Lys Val
85 90 95

Ser Gly Pro Ala Ala Pro Ala Gln Ser Tyr Thr Ala Leu Phe Arg Thr
100 105 110

```
Gln Thr Ala Pro Gly Gly Gln Gly Ala Pro Trp Ala Glu Glu Leu Leu
        115                 120                 125

Ala Gly Trp Gln Ser Ala Glu Leu Gly Gly Leu His Trp Gly Gln Asp
    130                 135                 140

Tyr Glu Phe Lys Val Arg Pro Ser Ser Gly Arg Ala Arg Gly Pro Asp
145                 150                 155                 160

Ser Asn Val Leu Leu Leu Arg Leu Pro Glu Lys Val Pro Ser Ala Pro
                165                 170                 175

Pro Gln Glu Val Thr Leu Lys Pro Gly Asn Gly Thr Val Phe Val Ser
            180                 185                 190

Trp Val Pro Pro Pro Ala Glu Asn His Asn Gly Ile Ile Arg Gly Tyr
        195                 200                 205

Gln Val Trp Ser Leu Gly Asn Thr Ser Leu Pro Pro Ala Asn Trp Thr
    210                 215                 220

Val Val Gly Glu Gln Thr Gln Leu Glu Ile Ala Thr His Met Pro Gly
225                 230                 235                 240

Ser Tyr Cys Val Gln Val Ala Ala Val Thr Gly Ala Gly Ala Gly Glu
                245                 250                 255

Pro Ser Arg Pro Val Cys Leu Leu Leu Glu Gln Ala Met Glu Arg Ala
            260                 265                 270

Thr Gln Glu Pro Ser Glu His Gly Pro Trp Thr Leu Glu Gln Leu Arg
        275                 280                 285

Ala Thr Leu Lys Arg Pro Glu Val Ile Ala Thr Cys Gly Val Ala Leu
    290                 295                 300

Trp Leu Leu Leu Leu Gly Thr Ala Val Cys Ile His Arg Arg Arg Arg
305                 310                 315                 320

Ala Arg Val His Leu Gly Pro Gly Leu Tyr Arg Tyr Thr Ser Glu Asp
                325                 330                 335

Ala Ile Leu Lys His Arg Met Asp His Ser Asp Ser Gln Trp Leu Ala
            340                 345                 350

Asp Thr Trp Arg Ser Thr Ser Gly Ser Arg Asp Leu Ser Ser Ser Ser
        355                 360                 365

Ser Leu Ser Ser Arg Leu Gly Ala Asp Ala Arg Asp Pro Leu Asp Cys
    370                 375                 380
```

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Arg Arg Ser Leu Leu Ser Trp Asp Ser Arg Ser Pro Gly Val Pro Leu
385                 390                 395                 400

Leu Pro Asp Thr Ser Thr Phe Tyr Gly Ser Leu Ile Ala Glu Leu Pro
                405                 410                 415

Ser Ser Thr Pro Ala Arg Pro Ser Pro Gln Val Pro Ala Val Arg Arg
            420                 425                 430

Leu Pro Pro Gln Leu Ala Gln Leu Ser Ser Pro Cys Ser Ser Ser Asp
        435                 440                 445

Ser Leu Cys Ser Arg Arg Gly Leu Ser Ser Pro Arg Leu Ser Leu Ala
    450                 455                 460

Pro Ala Glu Ala Trp Lys Ala Lys Lys Lys Gln Glu Leu Gln His Ala
465                 470                 475                 480

Asn Ser Ser Pro Leu Leu Arg Gly Ser His Ser Leu Glu Leu Arg Ala
                485                 490                 495

Cys Glu Leu Gly Asn Arg Gly Ser Lys Asn Leu Ser Gln Ser Pro Gly
            500                 505                 510

Ala Val Pro Gln Ala Leu Val Ala Trp Arg Ala Leu Gly Pro Lys Leu
        515                 520                 525

Leu Ser Ser Ser Asn Glu Leu Val Thr Arg His Leu Pro Pro Ala Pro
    530                 535                 540

Leu Phe Pro His Glu Thr Pro Pro Thr Gln Ser Gln Gln Thr Gln Pro
545                 550                 555                 560

Pro Val Ala Pro Gln Ala Pro Ser Ser Ile Leu Leu Pro Ala Ala Pro
                565                 570                 575

Ile Pro Ile Leu Ser Pro Cys Ser Pro Pro Ser Pro Gln Ala Ser Ser
            580                 585                 590

Leu Ser Gly Pro Ser Pro Ala Ser Ser Arg Leu Ser Ser Ser Ser Leu
        595                 600                 605

Ser Ser Leu Gly Glu Asp Gln Asp Ser Val Leu Thr Pro Glu Glu Val
    610                 615                 620

Ala Leu Cys Leu Glu Leu Ser Glu Gly Glu Glu Thr Pro Arg Asn Ser
625                 630                 635                 640

Val Ser Pro Met Pro Arg Ala Pro Ser Pro Pro Thr Thr Tyr Gly Tyr
                645                 650                 655
```

Ile Ser Val Pro Thr Ala Ser Glu Phe Thr Asp Met Gly Arg Thr Gly
660 665 670

Gly Gly Val Gly Pro Lys Gly Gly Val Leu Leu Cys Pro Pro Arg Pro
675 680 685

Cys Leu Thr Pro Thr Pro Ser Glu Gly Ser Leu Ala Asn Gly Trp Gly
690 695 700

Ser Ala Ser Glu Asp Asn Ala Ala Ser Ala Arg Ala Ser Leu Val Ser
705 710 715 720

Ser Ser Asp Gly Ser Phe Leu Ala Asp Ala His Phe Ala Arg Ala Leu
725 730 735

Ala Val Ala Val Asp Ser Phe Gly Phe Gly Leu Glu Pro Arg Glu Ala
740 745 750

Asp Cys Val Phe Ile Asp Ala Ser Ser Pro Pro Ser Pro Arg Asp Glu
755 760 765

Ile Phe Leu Thr Pro Asn Leu Ser Leu Pro Leu Trp Glu Trp Arg Pro
770 775 780

Asp Trp Leu Glu Asp Met Glu Val Ser His Thr Gln Arg Leu Gly Arg
785 790 795 800

Gly Met Pro Pro Trp Pro Pro Asp Ser Gln Ile Ser Ser Gln Arg Ser
805 810 815

Gln Leu His Cys Arg Met Pro Lys Ala Gly Ala Ser Pro Val Asp Tyr
820 825 830

Ser Ala Ser
835

<210> 11
<211> 2454
<212> DNA
<213> Artificial Sequence

<220>
<223> FLAG-hROBO4-225na

<400> 11
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ctcatcatgg gaggcatggc tgattacaag gatgacgacg ataagatcca ggagccccag 120

gactacacgg agcctgtgga gcttctggct gtgcgaattc agctggaaaa tgtgacactg 180

ctgaacccgg atcctgcaga gggccccaag cctagaccgg cggtgtggct cagctggaag 240

gtcagtggcc ctgctgcgcc tgcccaatct tacacggcct tgttcaggac ccagactgcc 300
ccgggaggcc agggagctcc gtgggcagag gagctgctgg ccggctggca gagcgcagag 360
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acccatatgc caggctccta ctgcgtgcaa gtggctgcag tcactggtgc tggagctggg 720
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caccgccggc gccgagctag ggtgcacctg ggcccaggtc tgtacagata taccagtgag 960
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caagctctgg ttgcctggcg ggccctggga ccgaaactcc tcagctcctc aaatgagctg 1560
gttactcgtc atctccctcc agcacccctc tttcctcatg aaactccccc aactcagagt 1620
caacagaccc agcctccggt ggcaccacag gctccctcct ccatcctgct gccagcagcc 1680
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ctggcagtgg ctgtggatag ctttggtttc ggtctagagc ccagggaggc agactgcgtc 2220
ttcatagatg cctcatcacc tccctcccca cgggatgaga tcttcctgac ccccaacctc 2280

tccctgcccc tgtgggagtg gaggccagac tggttggaag acatggaggt cagccacacc 2340

cagcggctgg gaaaggggat gcctccctgg ccccctgact ctcagatctc ttcccagaga 2400

agtcagctcc actgtcgtat gcccaaggct ggtgcttctc ctgtagatta ctcc 2454

<210> 12
<211> 818
<212> PRT
<213> Artificial Sequence

<220>
<223> FLAG-hROBO4-225aa

<400> 12

Met Gly Ser Gly Gly Asp Ser Leu Leu Gly Gly Arg Gly Ser Leu Pro
1 5 10 15

Leu Leu Leu Leu Leu Ile Met Gly Gly Met Ala Asp Tyr Lys Asp Asp
20 25 30

Asp Asp Lys Ile Gln Glu Pro Gln Asp Tyr Thr Glu Pro Val Glu Leu
35 40 45

Leu Ala Val Arg Ile Gln Leu Glu Asn Val Thr Leu Leu Asn Pro Asp
50 55 60

Pro Ala Glu Gly Pro Lys Pro Arg Pro Ala Val Trp Leu Ser Trp Lys
65 70 75 80

Val Ser Gly Pro Ala Ala Pro Ala Gln Ser Tyr Thr Ala Leu Phe Arg
85 90 95

Thr Gln Thr Ala Pro Gly Gly Gln Gly Ala Pro Trp Ala Glu Glu Leu
100 105 110

Leu Ala Gly Trp Gln Ser Ala Glu Leu Gly Gly Leu His Trp Gly Gln
115 120 125

Asp Tyr Glu Phe Lys Val Arg Pro Ser Ser Gly Arg Ala Arg Gly Pro
130 135 140

Asp Ser Asn Val Leu Leu Leu Arg Leu Pro Glu Lys Val Pro Ser Ala
145 150 155 160

Pro Pro Gln Glu Val Thr Leu Lys Pro Gly Asn Gly Thr Val Phe Val
165 170 175

Ser Trp Val Pro Pro Pro Ala Glu Asn His Asn Gly Ile Ile Arg Gly
180 185 190

Tyr Gln Val Trp Ser Leu Gly Asn Thr Ser Leu Pro Pro Ala Asn Trp

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        195                 200                 205

Thr Val Val Gly Glu Gln Thr Gln Leu Glu Ile Ala Thr His Met Pro
    210                 215                 220

Gly Ser Tyr Cys Val Gln Val Ala Ala Val Thr Gly Ala Gly Ala Gly
225                 230                 235                 240

Glu Pro Ser Arg Pro Val Cys Leu Leu Leu Glu Gln Ala Met Glu Arg
                245                 250                 255

Ala Thr Gln Glu Pro Ser Glu His Gly Pro Trp Thr Leu Glu Gln Leu
            260                 265                 270

Arg Ala Thr Leu Lys Arg Pro Glu Val Ile Ala Thr Cys Gly Val Ala
        275                 280                 285

Leu Trp Leu Leu Leu Leu Gly Thr Ala Val Cys Ile His Arg Arg Arg
    290                 295                 300

Arg Ala Arg Val His Leu Gly Pro Gly Leu Tyr Arg Tyr Thr Ser Glu
305                 310                 315                 320

Asp Ala Ile Leu Lys His Arg Met Asp His Ser Asp Ser Gln Trp Leu
                325                 330                 335

Ala Asp Thr Trp Arg Ser Thr Ser Gly Ser Arg Asp Leu Ser Ser Ser
            340                 345                 350

Ser Ser Leu Ser Ser Arg Leu Gly Ala Asp Ala Arg Asp Pro Leu Asp
        355                 360                 365

Cys Arg Arg Ser Leu Leu Ser Trp Asp Ser Arg Ser Pro Gly Val Pro
    370                 375                 380

Leu Leu Pro Asp Thr Ser Thr Phe Tyr Gly Ser Leu Ile Ala Glu Leu
385                 390                 395                 400

Pro Ser Ser Thr Pro Ala Arg Pro Ser Pro Gln Val Pro Ala Val Arg
                405                 410                 415

Arg Leu Pro Pro Gln Leu Ala Gln Leu Ser Ser Pro Cys Ser Ser Ser
            420                 425                 430

Asp Ser Leu Cys Ser Arg Arg Gly Leu Ser Ser Pro Arg Leu Ser Leu
        435                 440                 445

Ala Pro Ala Glu Ala Trp Lys Ala Lys Lys Lys Gln Glu Leu Gln His
    450                 455                 460
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Ala Asn Ser Ser Pro Leu Leu Arg Gly Ser His Ser Leu Glu Leu Arg
465                 470                 475                 480

Ala Cys Glu Leu Gly Asn Arg Gly Ser Lys Asn Leu Ser Gln Ser Pro
                485                 490                 495

Gly Ala Val Pro Gln Ala Leu Val Ala Trp Arg Ala Leu Gly Pro Lys
            500                 505                 510

Leu Leu Ser Ser Ser Asn Glu Leu Val Thr Arg His Leu Pro Pro Ala
        515                 520                 525

Pro Leu Phe Pro His Glu Thr Pro Pro Thr Gln Ser Gln Gln Thr Gln
    530                 535                 540

Pro Pro Val Ala Pro Gln Ala Pro Ser Ser Ile Leu Leu Pro Ala Ala
545                 550                 555                 560

Pro Ile Pro Ile Leu Ser Pro Cys Ser Pro Pro Ser Pro Gln Ala Ser
                565                 570                 575

Ser Leu Ser Gly Pro Ser Pro Ala Ser Ser Arg Leu Ser Ser Ser Ser
            580                 585                 590

Leu Ser Ser Leu Gly Glu Asp Gln Asp Ser Val Leu Thr Pro Glu Glu
        595                 600                 605

Val Ala Leu Cys Leu Glu Leu Ser Glu Gly Glu Glu Thr Pro Arg Asn
    610                 615                 620

Ser Val Ser Pro Met Pro Arg Ala Pro Ser Pro Pro Thr Thr Tyr Gly
625                 630                 635                 640

Tyr Ile Ser Val Pro Thr Ala Ser Glu Phe Thr Asp Met Gly Arg Thr
                645                 650                 655

Gly Gly Gly Val Gly Pro Lys Gly Gly Val Leu Leu Cys Pro Pro Arg
            660                 665                 670

Pro Cys Leu Thr Pro Thr Pro Ser Glu Gly Ser Leu Ala Asn Gly Trp
        675                 680                 685

Gly Ser Ala Ser Glu Asp Asn Ala Ala Ser Ala Arg Ala Ser Leu Val
    690                 695                 700

Ser Ser Ser Asp Gly Ser Phe Leu Ala Asp Ala His Phe Ala Arg Ala
705                 710                 715                 720

Leu Ala Val Ala Val Asp Ser Phe Gly Phe Gly Leu Glu Pro Arg Glu
                725                 730                 735
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Ala Asp Cys Val Phe Ile Asp Ala Ser Ser Pro Pro Ser Pro Arg Asp
740 745 750

Glu Ile Phe Leu Thr Pro Asn Leu Ser Leu Pro Leu Trp Glu Trp Arg
755 760 765

Pro Asp Trp Leu Glu Asp Met Glu Val Ser His Thr Gln Arg Leu Gly
770 775 780

Arg Gly Met Pro Pro Trp Pro Pro Asp Ser Gln Ile Ser Ser Gln Arg
785 790 795 800

Ser Gln Leu His Cys Arg Met Pro Lys Ala Gly Ala Ser Pro Val Asp
805 810 815

Tyr Ser

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<212> DNA
<213> Artificial Sequence

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gtgcccagtg ccccacctca ggaagtgact ctaaagcctg gcaatggcac tgtctttgtg 180

agctgggtcc caccacctgc tgaaaaccac aatggcatca tccgtggcta ccaggtctgg 240

agcctgggca acacatcact gccaccagcc aactggactg tagttggtga gcagacccag 300

ctggaaatcg ccacccatat gccaggctcc tactgcgtgc aagtggctgc agtcactggt 360

gctggagctg gggagcccag tagacctgtc tgcctccttt tagagcaggc catggagcga 420

gccacccaag aacccagtga gcatggtccc tggaccctgg agcagctgag ggctaccttg 480

aagcggcctg aggtcattgc cacctgcggt gttgcactct ggctgctgct tctgggcacc 540

gccgtgtgta tccaccgccg gcgccgagct agggtgcacc tgggcccagg tctgtacaga 600

tataccagtg aggatgccat cctaaaacac aggatggatc acagtgactc ccagtggttg 660

gcagacactt ggcgttccac ctctggctct cgggacctga gcagcagcag cagcctcagc 720

agtcggctgg gggcggatgc ccgggaccca ctagactgtc gtcgctcctt gctctcctgg 780

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<210> 14
<211> 702
<212> PRT
<213> Artificial Sequence

<220>
<223> FLAG-hROBO4-341aa

<400> 14

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Asp Asp Lys Arg Leu Pro Glu Lys Val Pro Ser Ala Pro Pro Gln Glu
35 40 45

Val Thr Leu Lys Pro Gly Asn Gly Thr Val Phe Val Ser Trp Val Pro
50 55 60

Pro Pro Ala Glu Asn His Asn Gly Ile Ile Arg Gly Tyr Gln Val Trp

65 70 75 80

Ser Leu Gly Asn Thr Ser Leu Pro Pro Ala Asn Trp Thr Val Val Gly
85 90 95

Glu Gln Thr Gln Leu Glu Ile Ala Thr His Met Pro Gly Ser Tyr Cys
100 105 110

Val Gln Val Ala Ala Val Thr Gly Ala Gly Ala Gly Glu Pro Ser Arg
115 120 125

Pro Val Cys Leu Leu Leu Glu Gln Ala Met Glu Arg Ala Thr Gln Glu
130 135 140

Pro Ser Glu His Gly Pro Trp Thr Leu Glu Gln Leu Arg Ala Thr Leu
145 150 155 160

Lys Arg Pro Glu Val Ile Ala Thr Cys Gly Val Ala Leu Trp Leu Leu
165 170 175

Leu Leu Gly Thr Ala Val Cys Ile His Arg Arg Arg Arg Ala Arg Val
180 185 190

His Leu Gly Pro Gly Leu Tyr Arg Tyr Thr Ser Glu Asp Ala Ile Leu
195 200 205

Lys His Arg Met Asp His Ser Asp Ser Gln Trp Leu Ala Asp Thr Trp
210 215 220

Arg Ser Thr Ser Gly Ser Arg Asp Leu Ser Ser Ser Ser Ser Leu Ser
225 230 235 240

Ser Arg Leu Gly Ala Asp Ala Arg Asp Pro Leu Asp Cys Arg Arg Ser
245 250 255

Leu Leu Ser Trp Asp Ser Arg Ser Pro Gly Val Pro Leu Leu Pro Asp
260 265 270

Thr Ser Thr Phe Tyr Gly Ser Leu Ile Ala Glu Leu Pro Ser Ser Thr
275 280 285

Pro Ala Arg Pro Ser Pro Gln Val Pro Ala Val Arg Arg Leu Pro Pro
290 295 300

Gln Leu Ala Gln Leu Ser Ser Pro Cys Ser Ser Ser Asp Ser Leu Cys
305 310 315 320

Ser Arg Arg Gly Leu Ser Ser Pro Arg Leu Ser Leu Ala Pro Ala Glu
325 330 335

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Ala Trp Lys Ala Lys Lys Lys Gln Glu Leu Gln His Ala Asn Ser Ser
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Pro Leu Leu Arg Gly Ser His Ser Leu Glu Leu Arg Ala Cys Glu Leu
        355                 360                 365

Gly Asn Arg Gly Ser Lys Asn Leu Ser Gln Ser Pro Gly Ala Val Pro
    370                 375                 380

Gln Ala Leu Val Ala Trp Arg Ala Leu Gly Pro Lys Leu Leu Ser Ser
385                 390                 395                 400

Ser Asn Glu Leu Val Thr Arg His Leu Pro Pro Ala Pro Leu Phe Pro
                405                 410                 415

His Glu Thr Pro Pro Thr Gln Ser Gln Gln Thr Gln Pro Pro Val Ala
            420                 425                 430

Pro Gln Ala Pro Ser Ser Ile Leu Leu Pro Ala Ala Pro Ile Pro Ile
        435                 440                 445

Leu Ser Pro Cys Ser Pro Pro Ser Pro Gln Ala Ser Ser Leu Ser Gly
    450                 455                 460

Pro Ser Pro Ala Ser Ser Arg Leu Ser Ser Ser Ser Leu Ser Ser Leu
465                 470                 475                 480

Gly Glu Asp Gln Asp Ser Val Leu Thr Pro Glu Glu Val Ala Leu Cys
                485                 490                 495

Leu Glu Leu Ser Glu Gly Glu Glu Thr Pro Arg Asn Ser Val Ser Pro
            500                 505                 510

Met Pro Arg Ala Pro Ser Pro Pro Thr Thr Tyr Gly Tyr Ile Ser Val
        515                 520                 525

Pro Thr Ala Ser Glu Phe Thr Asp Met Gly Arg Thr Gly Gly Gly Val
    530                 535                 540

Gly Pro Lys Gly Gly Val Leu Leu Cys Pro Pro Arg Pro Cys Leu Thr
545                 550                 555                 560

Pro Thr Pro Ser Glu Gly Ser Leu Ala Asn Gly Trp Gly Ser Ala Ser
                565                 570                 575

Glu Asp Asn Ala Ala Ser Ala Arg Ala Ser Leu Val Ser Ser Ser Asp
            580                 585                 590

Gly Ser Phe Leu Ala Asp Ala His Phe Ala Arg Ala Leu Ala Val Ala
        595                 600                 605
```

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Val Asp Ser Phe Gly Phe Gly Leu Glu Pro Arg Glu Ala Asp Cys Val
    610                 615                 620

Phe Ile Asp Ala Ser Ser Pro Pro Ser Pro Arg Asp Glu Ile Phe Leu
625                 630                 635                 640

Thr Pro Asn Leu Ser Leu Pro Leu Trp Glu Trp Arg Pro Asp Trp Leu
                645                 650                 655

Glu Asp Met Glu Val Ser His Thr Gln Arg Leu Gly Arg Gly Met Pro
            660                 665                 670

Pro Trp Pro Pro Asp Ser Gln Ile Ser Ser Gln Arg Ser Gln Leu His
        675                 680                 685

Cys Arg Met Pro Lys Ala Gly Ala Ser Pro Val Asp Tyr Ser
    690                 695                 700
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<210> 15
<211> 3045
<212> DNA
<213> Mus musculus

<400> 15

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gtgggagaga gcttggttct tgagtgtggt cctccctggg gctacccaaa accctcggtc      540

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gattccctga tggtgtcaag agcagagaag aatgactcgg ggacctatat gtgtatggcc      660

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ctaaaccccg aacctgtaaa aggtcccaag cctgggccat ccgtgtggct cagctggaag      840

gtgagcggcc ctgctgcacc tgctgagtca tacacagctc tgttcaggac tcagaggtcc      900

cccagggacc aaggatctcc atggacagag gtgctgctgc gtggcttgca gagtgcaaag      960

cttgggggtc tccactgggg ccaagactat gaattcaaag tgagaccgtc ctccggccgg     1020

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<210> 16
<211> 1015
<212> PRT
<213> Mus musculus

<400> 16

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Gly Leu Leu Gly Thr Glu Trp Pro Leu Pro Leu Leu Leu Leu Phe Ile
20 25 30

Met Gly Gly Glu Ala Leu Asp Ser Pro Pro Gln Ile Leu Val His Pro
35 40 45

Gln Asp Gln Leu Leu Gln Gly Ser Gly Pro Ala Lys Met Arg Cys Arg
50 55 60

Ser Ser Gly Gln Pro Pro Pro Thr Ile Arg Trp Leu Leu Asn Gly Gln
65 70 75 80

Pro Leu Ser Met Ala Thr Pro Asp Leu His Tyr Leu Leu Pro Asp Gly
85 90 95

Thr Leu Leu Leu His Arg Pro Ser Val Gln Gly Arg Pro Gln Asp Asp
100 105 110

Gln Asn Ile Leu Ser Ala Ile Leu Gly Val Tyr Thr Cys Glu Ala Ser
115 120 125

Asn Arg Leu Gly Thr Ala Val Ser Arg Gly Ala Arg Leu Ser Val Ala
130 135 140

Val Leu Gln Glu Asp Phe Gln Ile Gln Pro Arg Asp Thr Val Ala Val
145 150 155 160

Val Gly Glu Ser Leu Val Leu Glu Cys Gly Pro Pro Trp Gly Tyr Pro
165 170 175

Lys Pro Ser Val Ser Trp Trp Lys Asp Gly Lys Pro Leu Val Leu Gln
180 185 190

Pro Gly Arg Arg Thr Val Ser Gly Asp Ser Leu Met Val Ser Arg Ala
195 200 205

Glu Lys Asn Asp Ser Gly Thr Tyr Met Cys Met Ala Thr Asn Asn Ala
210 215 220

Gly Gln Arg Glu Ser Arg Ala Ala Arg Val Ser Ile Gln Glu Ser Gln
225 230 235 240

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Asp His Lys Glu His Leu Glu Leu Leu Ala Val Arg Ile Gln Leu Glu
                245                 250                 255

Asn Val Thr Leu Leu Asn Pro Glu Pro Val Lys Gly Pro Lys Pro Gly
            260                 265                 270

Pro Ser Val Trp Leu Ser Trp Lys Val Ser Gly Pro Ala Ala Pro Ala
        275                 280                 285

Glu Ser Tyr Thr Ala Leu Phe Arg Thr Gln Arg Ser Pro Arg Asp Gln
    290                 295                 300

Gly Ser Pro Trp Thr Glu Val Leu Leu Arg Gly Leu Gln Ser Ala Lys
305                 310                 315                 320

Leu Gly Gly Leu His Trp Gly Gln Asp Tyr Glu Phe Lys Val Arg Pro
                325                 330                 335

Ser Ser Gly Arg Ala Arg Gly Pro Asp Ser Asn Val Leu Leu Leu Arg
            340                 345                 350

Leu Pro Glu Gln Val Pro Ser Ala Pro Pro Gln Gly Val Thr Leu Arg
        355                 360                 365

Ser Gly Asn Gly Ser Val Phe Val Ser Trp Ala Pro Pro Pro Ala Glu
    370                 375                 380

Ser His Asn Gly Val Ile Arg Gly Tyr Gln Val Trp Ser Leu Gly Asn
385                 390                 395                 400

Ala Ser Leu Pro Ala Ala Asn Trp Thr Val Val Gly Glu Gln Thr Gln
                405                 410                 415

Leu Glu Ile Ala Thr Arg Leu Pro Gly Ser Tyr Cys Val Gln Val Ala
            420                 425                 430

Ala Val Thr Gly Ala Gly Ala Gly Glu Leu Ser Thr Pro Val Cys Leu
        435                 440                 445

Leu Leu Glu Gln Ala Met Glu Gln Ser Ala Arg Asp Pro Arg Lys His
    450                 455                 460

Val Pro Trp Thr Leu Glu Gln Leu Arg Ala Thr Leu Arg Arg Pro Glu
465                 470                 475                 480

Val Ile Ala Ser Ser Ala Val Leu Leu Trp Leu Leu Leu Leu Gly Ile
                485                 490                 495

Thr Val Cys Ile Tyr Arg Arg Arg Lys Ala Gly Val His Leu Gly Pro
            500                 505                 510
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Gly Leu Tyr Arg Tyr Thr Ser Glu Asp Ala Ile Leu Lys His Arg Met
        515                 520                 525

Asp His Ser Asp Ser Pro Trp Leu Ala Asp Thr Trp Arg Ser Thr Ser
    530                 535                 540

Gly Ser Arg Asp Leu Ser Ser Ser Ser Ser Leu Ser Ser Arg Leu Gly
545                 550                 555                 560

Leu Asp Pro Arg Asp Pro Leu Glu Gly Arg Arg Ser Leu Ile Ser Trp
                565                 570                 575

Asp Pro Arg Ser Pro Gly Val Pro Leu Leu Pro Asp Thr Ser Thr Phe
            580                 585                 590

Tyr Gly Ser Leu Ile Ala Glu Gln Pro Ser Ser Pro Pro Val Arg Pro
        595                 600                 605

Ser Pro Lys Thr Pro Ala Ala Arg Arg Phe Pro Ser Lys Leu Ala Gly
    610                 615                 620

Thr Ser Ser Pro Trp Ala Ser Ser Asp Ser Leu Cys Ser Arg Arg Gly
625                 630                 635                 640

Leu Cys Ser Pro Arg Met Ser Leu Thr Pro Thr Glu Ala Trp Lys Ala
                645                 650                 655

Lys Lys Lys Gln Glu Leu His Gln Ala Asn Ser Ser Pro Leu Leu Arg
            660                 665                 670

Gly Ser His Pro Met Glu Ile Trp Ala Trp Glu Leu Gly Ser Arg Ala
        675                 680                 685

Ser Lys Asn Leu Ser Gln Ser Pro Gly Glu Ala Pro Arg Ala Val Val
    690                 695                 700

Ser Trp Arg Ala Val Gly Pro Gln Leu His Arg Asn Ser Ser Glu Leu
705                 710                 715                 720

Ala Ser Arg Pro Leu Pro Pro Thr Pro Leu Ser Leu Arg Gly Ala Ser
                725                 730                 735

Ser His Asp Pro Gln Ser Gln Cys Val Glu Lys Leu Gln Ala Pro Ser
            740                 745                 750

Ser Asp Pro Leu Pro Ala Ala Pro Leu Ser Val Leu Asn Ser Ser Arg
        755                 760                 765

Pro Ser Ser Pro Gln Ala Ser Phe Leu Ser Cys Pro Ser Pro Ser Ser
    770                 775                 780
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Ser Asn Leu Ser Ser Ser Ser Leu Ser Ser Leu Glu Glu Glu Glu Asp
785                 790                 795                 800

Gln Asp Ser Val Leu Thr Pro Glu Glu Val Ala Leu Cys Leu Glu Leu
                805                 810                 815

Ser Asp Gly Glu Glu Thr Pro Thr Asn Ser Val Ser Pro Met Pro Arg
            820                 825                 830

Ala Pro Ser Pro Pro Thr Thr Tyr Gly Tyr Ile Ser Ile Pro Thr Cys
        835                 840                 845

Ser Gly Leu Ala Asp Met Gly Arg Ala Gly Gly Gly Val Gly Ser Glu
    850                 855                 860

Val Gly Asn Leu Leu Tyr Pro Pro Arg Pro Cys Pro Thr Pro Thr Pro
865                 870                 875                 880

Ser Glu Gly Ser Leu Ala Asn Gly Trp Gly Ser Ala Ser Glu Asp Asn
                885                 890                 895

Val Pro Ser Ala Arg Ala Ser Leu Val Ser Ser Ser Asp Gly Ser Phe
            900                 905                 910

Leu Ala Asp Thr His Phe Ala Arg Ala Leu Ala Val Ala Val Asp Ser
        915                 920                 925

Phe Gly Leu Ser Leu Asp Pro Arg Glu Ala Asp Cys Val Phe Thr Asp
    930                 935                 940

Ala Ser Ser Pro Pro Ser Pro Arg Gly Asp Leu Ser Leu Thr Arg Ser
945                 950                 955                 960

Phe Ser Leu Pro Leu Trp Glu Trp Arg Pro Asp Trp Leu Glu Asp Ala
                965                 970                 975

Glu Ile Ser His Thr Gln Arg Leu Gly Arg Gly Leu Pro Pro Trp Pro
            980                 985                 990

Pro Asp Ser Arg Ala Ser Ser Gln  Arg Ser Trp Leu Thr  Gly Ala Val
        995                 1000                1005

Pro Lys  Ala Gly Asp Ser Ser
    1010                1015
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<212> DNA
<213> Rattus norvegicus

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<212> PRT
<213> Rattus norvegicus

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20 25 30

Met Gly Gly Lys Ala Leu Asp Ser Pro Pro Gln Ile Leu Val His Pro
35 40 45

Gln Asp Gln Leu Leu Gln Gly Ser Gly Pro Ala Lys Met Ser Cys Arg
50 55 60

Ala Ser Gly Gln Pro Leu Pro Thr Ile Arg Trp Leu Leu Asn Gly Gln
65 70 75 80

Pro Leu Ser Met Ala Thr Pro Asp Leu His Tyr Leu Gln Ser Asp Gly

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                85                  90                  95

Thr Leu Leu Leu His Arg Pro Pro Thr His Gly Arg Pro Gln Asp Asp
            100                 105                 110

Gln Asn Ile Leu Ser Ala Ile Leu Gly Val Tyr Thr Cys Glu Ala Ser
        115                 120                 125

Asn Arg Leu Gly Thr Ala Val Ser Arg Gly Ala Arg Leu Ser Val Ala
    130                 135                 140

Val Leu Gln Glu Asp Phe Arg Ile Gln Pro Arg Asp Thr Val Ala Val
145                 150                 155                 160

Val Gly Glu Ser Leu Val Leu Glu Cys Gly Pro Pro Trp Gly Tyr Pro
                165                 170                 175

Lys Pro Ser Val Ser Trp Trp Lys Asp Gly Lys Pro Leu Val Leu Gln
            180                 185                 190

Pro Gly Lys Arg Thr Val Ser Gly Asp Ser Leu Met Val Ala Arg Ala
        195                 200                 205

Glu Lys Asn Asp Thr Gly Thr Tyr Met Cys Met Ala Thr Asn Asn Ala
    210                 215                 220

Gly Gln Arg Glu Ser Arg Ala Ala Arg Val Ser Ile Gln Glu Ser Pro
225                 230                 235                 240

Asp His Lys Glu His Leu Glu Leu Leu Ala Val Arg Ile Gln Leu Glu
                245                 250                 255

Asn Val Thr Leu Leu Asn Pro Glu Pro Val Lys Gly Pro Lys Pro Gly
            260                 265                 270

Pro Ala Val Trp Leu Ser Trp Lys Val Ser Gly Pro Ala Ala Pro Ala
        275                 280                 285

Gln Ser Tyr Thr Ala Leu Phe Arg Ala Gln Arg Asp Pro Arg Asp Gln
    290                 295                 300

Gly Ser Pro Trp Thr Glu Val Leu Leu Asp Gly Leu Leu Asn Ala Lys
305                 310                 315                 320

Leu Gly Gly Leu Arg Trp Gly Gln Asp Tyr Glu Phe Lys Val Arg Pro
                325                 330                 335

Ser Ser Gly Arg Ala Arg Gly Pro Asp Ser Asn Val Leu Leu Leu Arg
            340                 345                 350
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Leu Pro Glu Gln Val Pro Ser Ala Pro Pro Gln Glu Val Thr Leu Arg
        355                 360                 365

Pro Gly Asn Gly Ser Val Phe Val Ser Trp Ala Pro Pro Pro Ala Glu
    370                 375                 380

Asn His Asn Gly Phe Ile Arg Gly Tyr Gln Val Trp Ser Leu Gly Asn
385                 390                 395                 400

Ala Ser Leu Pro Ala Ala Asn Trp Thr Val Val Gly Glu Gln Thr Gln
                405                 410                 415

Leu Glu Ile Ala Ala Arg Met Pro Gly Ser Tyr Cys Val Gln Val Ala
            420                 425                 430

Ala Val Thr Gly Ala Gly Ala Gly Glu Pro Ser Ile Pro Val Cys Leu
        435                 440                 445

Leu Leu Glu Gln Ala Met Glu Gln Ser Ala Arg Asp Pro Ser Lys His
    450                 455                 460

Val Ser Trp Thr Leu Glu Gln Leu Arg Ala Thr Leu Lys Arg Pro Glu
465                 470                 475                 480

Val Ile Ala Ser Gly Ala Val Leu Leu Trp Leu Leu Leu Leu Gly Ile
                485                 490                 495

Ala Val Cys Ile Tyr Arg Arg Arg Lys Ala Gly Val His Leu Gly Pro
            500                 505                 510

Gly Leu Tyr Arg Tyr Thr Ser Glu Asp Ala Ile Leu Lys His Arg Met
        515                 520                 525

Asp His Ser Asp Ser Pro Trp Leu Ala Asp Thr Trp Arg Ser Thr Ser
    530                 535                 540

Gly Ser Arg Asp Leu Ser Ser Ser Ser Ser Leu Ser Ser Arg Leu Gly
545                 550                 555                 560

Val Asp Pro Arg Asp Pro Leu Asp Gly Arg Arg Ser Leu Ile Ser Trp
                565                 570                 575

Asp Pro Arg Ser Pro Gly Val Pro Leu Leu Pro Asp Thr Ser Thr Phe
            580                 585                 590

Tyr Gly Ser Leu Ile Ala Glu Gln Thr Ser Ser Pro Pro Val Arg Pro
        595                 600                 605

Ser Pro Gln Thr Pro Ala Ala Arg Arg Leu Pro Pro Lys Leu Thr Gly
    610                 615                 620
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Thr Ser Ser Pro Trp Ala Ser Ser Asp Ser Leu Cys Ser Arg Arg Gly
625                 630                 635                 640

Leu Cys Ser Pro Arg Met Ser Leu Ala Pro Ala Glu Ala Trp Lys Ala
                645                 650                 655

Lys Lys Lys Gln Glu Leu His Gln Ala Asn Ser Ser Pro Leu Leu Gln
            660                 665                 670

Gly Ser His Pro Met Glu Ile Trp Ala Trp Glu Leu Gly Ser Arg Ala
        675                 680                 685

Ser Lys Asn Leu Ser Gln Ser Pro Gly Pro Asn Thr Cys Ser Pro Arg
    690                 695                 700

Glu Ala Pro Gly Ala Val Val Ala Trp Arg Ala Leu Gly Pro Gln Leu
705                 710                 715                 720

His Arg Asn Ser Ser Glu Leu Ala Ala Arg Pro Leu Pro Pro Thr Pro
                725                 730                 735

Leu Ser Leu Arg Gly Ala Pro Ser His Asp Pro Gln Ser Gln Cys Val
            740                 745                 750

Glu Lys Leu Gln Ala Pro Ser Ser Asp Pro Leu Pro Ala Ala Pro Leu
        755                 760                 765

Ser Val Leu Asn Ser Ser Arg Pro Ser Ser Pro Gln Ala Ser Phe Leu
    770                 775                 780

Ser Val Pro Ser Pro Gly Ser Ser Asn Leu Ser Ser Ser Ser Leu Ser
785                 790                 795                 800

Ser Leu Glu Glu Glu Asp Gln Asp Ser Val Leu Thr Pro Glu Glu Val
                805                 810                 815

Ala Leu Cys Leu Glu Leu Ser Asp Gly Glu Glu Thr Pro Thr Asn Ser
            820                 825                 830

Val Ser Pro Met Pro Arg Ala Pro Ser Pro Pro Ala Thr Tyr Gly Tyr
        835                 840                 845

Ile Ser Ile Pro Thr Ser Ser Gly Leu Ala Asp Met Gly Arg Ala Gly
    850                 855                 860

Gly Gly Val Gly Ser Glu Val Gly Asn Leu Leu Cys Pro Pro Arg Leu
865                 870                 875                 880

Cys Pro Thr Pro Thr Pro Ser Glu Gly Ser Leu Ala Asn Gly Trp Gly
                885                 890                 895
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Ser Ala Ser Glu Asp Asn Val Pro Ser Ala Arg Ala Ser Leu Val Ser
            900                 905                 910

Ser Ser Asp Gly Ser Phe Leu Ala Asp Ala His Phe Ala Arg Ala Leu
        915                 920                 925

Ala Val Ala Val Asp Ser Phe Gly Phe Ser Leu Glu Pro Arg Glu Ala
    930                 935                 940

Asp Cys Val Phe Thr Asp Ala Ser Ser Pro Pro Ser Pro Arg Asp Asp
945                 950                 955                 960

Leu Ser Leu Thr Arg Ser Phe Ser Leu Pro Leu Trp Glu Trp Arg Pro
                965                 970                 975

Asp Trp Leu Glu Asp Ala Glu Ile Asn His Thr Gln Arg Leu Gly Arg
            980                 985                 990

Gly Leu Pro Pro Trp Pro Pro Asp  Ser Arg Val Ser Ser  Gln Arg Ser
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Trp Leu  Thr Gly Val Val Pro  Lys Ala Gly Asp Ser  Ser
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<213> Macaca fascicularis

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<213> Macaca fascicularis

<400> 20

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Gln Ile Leu Val His Pro Gln Asp Gln Leu Phe Gln Gly Pro Gly Pro
35 40 45

Ala Arg Met Ser Cys Arg Ala Ser Gly Gln Pro Pro Pro Thr Ile Arg
50 55 60

Trp Leu Leu Asn Gly Gln Pro Leu Ser Met Val Pro Pro Asp Pro His
65 70 75 80

His Leu Leu Pro Asp Gly Thr Leu Leu Leu Leu Gln Pro Pro Ala Trp
85 90 95

Gly His Ala His Asp Gly Gln Ala Leu Ser Thr Asp Leu Gly Val Tyr
100 105 110

Thr Cys Glu Ala Ser Asn Arg Leu Gly Thr Ala Val Ser Arg Gly Ala
115 120 125

Arg Leu Ser Val Ala Val Leu Arg Glu Asp Phe Gln Ile Gln Pro Arg
130 135 140

Asp Met Val Ala Val Val Gly Glu Gln Leu Thr Leu Glu Cys Gly Pro
145 150 155 160

Pro Trp Gly His Pro Glu Pro Thr Val Ser Trp Trp Lys Asp Gly Lys
165 170 175

Pro Leu Val Leu Gln Pro Gly Arg Tyr Thr Val Ser Gly Gly Ser Leu
180 185 190

Leu Met Ala Arg Ala Glu Lys Ser Asp Ala Gly Ala Tyr Met Cys Val
195 200 205

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Ala Ala Asn Ser Ala Gly His Arg Glu Ser His Ala Ala Arg Val Ser
    210                 215                 220

Ile Gln Glu Pro Gln Asp Tyr Thr Glu Pro Val Glu Leu Leu Ala Val
225                 230                 235                 240

Arg Ile Gln Leu Glu Asn Val Thr Leu Leu Asn Pro Asp Pro Ala Lys
                245                 250                 255

Gly Pro Lys Pro Gly Pro Ala Val Trp Leu Ser Trp Lys Val Ser Gly
            260                 265                 270

Pro Ala Ala Pro Ala Gln Ser Tyr Thr Ala Leu Phe Arg Thr Gln Thr
        275                 280                 285

Ala Pro Gly Asp Gln Gly Ala Pro Trp Thr Glu Glu Leu Leu Ala Gly
    290                 295                 300

Trp Gln Ser Ala Glu Leu Gly Gly Leu His Trp Gly Gln Asp Tyr Glu
305                 310                 315                 320

Phe Lys Val Arg Pro Ser Ser Gly Arg Ala Arg Gly Pro Asp Ser Asn
                325                 330                 335

Val Leu Leu Leu Arg Leu Pro Glu Lys Val Pro Ser Ala Pro Pro Gln
            340                 345                 350

Glu Val Thr Leu Lys Pro Gly Asn Gly Ser Val Leu Val Ser Trp Val
        355                 360                 365

Pro Pro Pro Ala Glu Asn His Asn Gly Ile Ile Arg Gly Tyr Gln Val
    370                 375                 380

Trp Ser Leu Gly Asn Thr Ser Leu Pro Pro Ala Asn Trp Thr Val Val
385                 390                 395                 400

Gly Glu Gln Thr Gln Leu Glu Ile Ala Thr Arg Met Pro Gly Ser Tyr
                405                 410                 415

Cys Val Gln Val Ala Ala Val Thr Gly Ala Gly Ala Gly Glu Pro Ser
            420                 425                 430

Ser Pro Val Cys Leu Leu Leu Glu Gln Ala Met Glu Arg Ala Thr Arg
        435                 440                 445

Glu Pro Ser Glu His Gly Pro Trp Thr Leu Glu Gln Leu Arg Ala Thr
    450                 455                 460

Leu Lys Arg Pro Glu Val Ile Ala Thr Cys Gly Val Val Leu Trp Leu
465                 470                 475                 480
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Leu Leu Leu Gly Thr Ala Val Cys Ile His Arg Arg Arg Arg Ala Gly
                485                 490                 495

Val His Leu Gly Pro Gly Leu Tyr Arg Tyr Thr Ser Lys Asp Ala Ile
            500                 505                 510

Leu Lys His Arg Met Asp His Ser Asp Ser Gln Trp Leu Ala Asp Thr
        515                 520                 525

Trp Arg Ser Thr Ser Gly Ser Arg Asp Leu Ser Ser Ser Ser Ser Leu
    530                 535                 540

Ser Ser Arg Leu Gly Ala Asp Pro Arg Asp Pro Leu Asp Cys Arg Arg
545                 550                 555                 560

Ser Leu Leu Ser Trp Asp Ser Arg Ser Pro Gly Val Pro Leu Leu Pro
                565                 570                 575

Asp Thr Ser Thr Phe Tyr Gly Ser Leu Ile Ala Glu Leu Pro Ser Ser
            580                 585                 590

Pro Pro Ala Arg Pro Ser Pro Gln Val Pro Ala Val Arg Arg Leu Pro
        595                 600                 605

Pro Gln Leu Ala Arg Leu Ser Ser Pro Cys Ser Ser Ser Asp Ser Leu
    610                 615                 620

Cys Ser His Arg Gly Phe Ser Ser Pro Arg Leu Ser Leu Ala Pro Thr
625                 630                 635                 640

Glu Ala Trp Lys Ala Lys Lys Lys Gln Glu Leu Gln His Val Asn Ser
                645                 650                 655

Ser Pro Leu Leu Arg Gly Ser Gln Pro Leu Glu Leu Arg Ala Cys Glu
            660                 665                 670

Leu Gly Asn Arg Gly Ser Lys Asn Leu Ser Gln Ser Pro Gly Ala Val
        675                 680                 685

Pro Gln Ala Leu Val Ala Trp Arg Ala Leu Gly Pro Lys Leu Leu Ser
    690                 695                 700

Ser Ser Asn Glu Leu Val Thr Arg Pro Leu Pro Pro Ala Pro Leu Phe
705                 710                 715                 720

Pro His Glu Thr Pro Gln Thr Gln Ser Gln Gln Thr Gln Pro Pro Val
                725                 730                 735

Ala Pro Gln Ala Pro Ser Ser Ile Leu Leu Ala Thr Ala Pro Ile Pro
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            740                 745                 750

Ile Leu Ser Pro Cys Ser Pro Pro Ser Pro Gln Ala Ser Ser Leu Ser
        755                 760                 765

Gly Pro Ser Pro Ala Ser Ser His Leu Ser Ser Ser Ser Leu Ser Ser
    770                 775                 780

Leu Gly Glu Asp Gln Asp Ser Val Leu Thr Pro Glu Glu Val Ala Leu
785                 790                 795                 800

Cys Leu Glu Leu Ser Glu Gly Glu Glu Thr Pro Arg Asn Ser Val Ser
                805                 810                 815

Pro Met Pro Arg Ala Pro Ser Pro Pro Thr Thr Tyr Gly Tyr Ile Ser
            820                 825                 830

Thr Pro Thr Ala Ser Glu Phe Thr Gly Met Asp Arg Thr Gly Gly Gly
        835                 840                 845

Val Gly Ser Glu Gly Gly Val Leu Leu Cys Pro Pro Arg Pro Cys Leu
    850                 855                 860

Thr Pro Thr Pro Ser Glu Gly Ser Leu Ala Asn Gly Trp Gly Ser Ala
865                 870                 875                 880

Ser Glu Asp Asn Ala Ala Ser Ala Arg Ala Ser Leu Val Ser Ser Ser
                885                 890                 895

Asp Gly Ser Phe Leu Ala Asp Ala His Phe Ala Arg Ala Leu Ala Val
            900                 905                 910

Ala Val Asp Ser Phe Gly Phe Gly Leu Glu Pro Arg Glu Ala Asp Cys
        915                 920                 925

Val Phe Thr Asp Ala Ser Ser Pro Pro Ser Pro Arg Asp Asp Ile Phe
    930                 935                 940

Leu Thr Pro Asn Leu Ser Leu Pro Leu Trp Glu Trp Arg Pro Asp Trp
945                 950                 955                 960

Leu Glu Asp Met Glu Val Asn His Thr Gln Leu Leu Gly Arg Gly Met
                965                 970                 975

Pro Pro Trp Ser Pro Asp Ser Arg Ile Ser Ser Gln Arg Ser Gln Leu
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His Cys Pro Val Pro Lys Ala Gly  Ala Ser Pro Val Asp  Tyr Ser
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<213> Macaca fascicularis

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Ala Arg Met Ser Cys Arg Ala Ser Gly Gln Pro Pro Pro Thr Ile Arg
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Trp Leu Leu Asn Gly Gln Pro Leu Ser Met Val Pro Pro Asp Pro His
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His Leu Leu Pro Asp Gly Thr Leu Leu Leu Gln Pro Pro Ala Arg
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Gly His Ala His Asp Gly Gln Ala Leu Ser Thr Asp Leu Gly Val Tyr
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Thr Cys Glu Ala Ser Asn Arg Leu Gly Thr Ala Val Ser Arg Gly Ala
        115                 120                 125

Arg Leu Ser Val Ala Val Leu Arg Glu Asp Phe Gln Ile Gln Pro Arg
    130                 135                 140

Asp Met Val Ala Val Val Gly Glu Gln Leu Thr Leu Glu Cys Gly Pro
145                 150                 155                 160

Pro Trp Gly His Pro Glu Pro Thr Val Ser Trp Trp Lys Asp Gly Lys
                165                 170                 175

Pro Leu Val Leu Gln Pro Gly Arg Tyr Thr Val Ser Gly Gly Ser Leu
            180                 185                 190

Leu Met Ala Arg Ala Glu Lys Ser Asp Thr Gly Ala Tyr Met Cys Val
        195                 200                 205

Ala Ala Asn Ser Ala Gly His Arg Glu Ser Arg Ala Ala Arg Val Ser
    210                 215                 220

Ile Gln Glu Pro Gln Asp Tyr Thr Glu Pro Val Glu Leu Leu Ala Val
225                 230                 235                 240

Arg Ile Gln Leu Glu Asn Val Thr Leu Leu Asn Pro Asp Pro Ala Lys
                245                 250                 255

Gly Pro Lys Pro Gly Pro Ala Val Trp Leu Ser Trp Lys Val Ser Gly
            260                 265                 270

Pro Ala Ala Pro Ala Gln Ser Tyr Thr Ala Leu Phe Arg Thr Gln Thr
        275                 280                 285

Ala Pro Gly Asp Gln Gly Ala Pro Trp Thr Glu Glu Leu Leu Ala Gly
    290                 295                 300

Trp Gln Ser Ala Glu Leu Gly Gly Leu His Trp Gly Gln Asp Tyr Glu
305                 310                 315                 320

Phe Lys Val Arg Pro Ser Ser Gly Arg Ala Arg Gly Pro Asp Ser Asn
                325                 330                 335

Val Leu Leu Leu Arg Leu Pro Glu Lys Val Pro Ser Ala Pro Pro Gln
```

```
            340                 345                 350

Glu Val Thr Leu Lys Pro Gly Asn Gly Ser Val Leu Val Ser Trp Val
        355                 360                 365

Pro Pro Pro Ala Glu Asn His Asn Gly Ile Ile Arg Gly Tyr Gln Val
    370                 375                 380

Trp Ser Leu Gly Asn Thr Ser Leu Pro Pro Ala Asn Trp Thr Val Val
385                 390                 395                 400

Gly Glu Gln Thr Gln Leu Glu Ile Ala Thr Arg Met Pro Gly Ser Tyr
                405                 410                 415

Cys Val Gln Val Ala Ala Val Thr Gly Ala Gly Ala Gly Glu Pro Ser
            420                 425                 430

Ser Pro Val Cys Leu Leu Leu Glu Gln Ala Met Glu Arg Ala Thr Arg
        435                 440                 445

Glu Leu Ser Glu His Gly Pro Trp Thr Leu Glu Gln Leu Arg Ala Thr
    450                 455                 460

Leu Lys Arg Pro Glu Val Ile Ala Thr Cys Gly Val Val Leu Trp Leu
465                 470                 475                 480

Leu Leu Leu Gly Thr Ala Val Cys Ile His Arg Arg Arg Arg Ala Gly
                485                 490                 495

Val His Leu Gly Pro Gly Leu Tyr Arg Tyr Thr Ser Lys Asp Ala Ile
            500                 505                 510

Leu Lys His Arg Met Asp His Ser Asp Ser Gln Trp Leu Ala Asp Thr
        515                 520                 525

Trp Arg Ser Thr Ser Gly Ser Arg Asp Leu Ser Ser Ser Ser Ser Leu
    530                 535                 540

Ser Ser Arg Leu Gly Ala Asp Pro Arg Asp Pro Leu Asp Cys Arg Arg
545                 550                 555                 560

Ser Leu Leu Ser Trp Asp Ser Arg Ser Pro Gly Val Pro Leu Leu Leu
                565                 570                 575

Asp Thr Ser Thr Phe Tyr Gly Ser Leu Ile Ala Glu Leu Pro Ser Ser
            580                 585                 590

Pro Leu Ala Arg Pro Ser Pro Gln Val Pro Ala Val Arg Arg Leu Pro
        595                 600                 605
```

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Pro Gln Leu Ala Arg Leu Ser Ser Pro Cys Ser Ser Ser Asp Ser Leu
    610                 615                 620

Cys Ser His Arg Gly Leu Ser Ser Pro Arg Leu Ser Leu Ala Pro Thr
625                 630                 635                 640

Glu Ala Trp Lys Ala Lys Lys Lys Gln Glu Leu Gln His Val Asn Ser
                645                 650                 655

Ser Pro Leu Leu Arg Gly Ser Gln Pro Leu Glu Leu Arg Ala Cys Glu
            660                 665                 670

Leu Gly Asn Arg Gly Ser Lys Asn Leu Ser Gln Ser Pro Gly Ala Val
        675                 680                 685

Pro Gln Ala Leu Val Ala Trp Arg Ala Leu Gly Pro Lys Leu Leu Ser
    690                 695                 700

Ser Ser Asn Glu Leu Val Thr Arg Pro Leu Pro Pro Ala Pro Leu Phe
705                 710                 715                 720

Pro His Glu Thr Pro Gln Thr Gln Ser Gln Gln Thr Gln Pro Pro Val
                725                 730                 735

Ala Pro Gln Ala Pro Ser Ser Ile Leu Leu Ala Thr Ala Pro Ile Pro
            740                 745                 750

Ile Leu Ser Pro Cys Ser Pro Pro Ser Pro Gln Ala Ser Ser Leu Ser
        755                 760                 765

Gly Pro Ser Pro Ala Ser Ser His Leu Ser Ser Ser Ser Leu Ser Ser
    770                 775                 780

Leu Gly Glu Asp Gln Asp Ser Val Leu Thr Pro Glu Glu Val Ala Leu
785                 790                 795                 800

Cys Leu Glu Leu Ser Glu Gly Glu Glu Thr Pro Arg Asn Ser Val Ser
                805                 810                 815

Pro Met Pro Arg Ala Pro Ser Pro Pro Thr Thr Tyr Gly Tyr Ile Ser
            820                 825                 830

Thr Pro Thr Ala Ser Glu Phe Thr Gly Met Asp Arg Thr Gly Gly Gly
        835                 840                 845

Val Gly Ser Glu Gly Gly Val Leu Leu Cys Pro Pro Arg Pro Cys Leu
    850                 855                 860

Thr Pro Thr Pro Ser Glu Gly Ser Leu Ala Asn Gly Trp Gly Ser Ala
865                 870                 875                 880
```

Ser Glu Asp Asn Ala Ala Ser Ala Arg Ala Ser Leu Val Ser Ser Ser
885 890 895

Asp Gly Ser Phe Leu Ala Asp Ala His Phe Ala Arg Ala Leu Ala Val
900 905 910

Ala Val Asp Ser Phe Gly Phe Gly Leu Glu Pro Arg Glu Ala Asp Cys
915 920 925

Val Phe Thr Asp Ala Ser Ser Pro Pro Ser Pro Arg Asp Asp Ile Phe
930 935 940

Leu Thr Pro Asn Leu Ser Leu Pro Leu Trp Glu Trp Arg Pro Asp Trp
945 950 955 960

Leu Glu Asp Met Glu Val Asn His Thr Gln Leu Leu Gly Arg Gly Met
965 970 975

Pro Pro Trp Ser Pro Asp Ser Arg Ile Ser Ser Gln Arg Ser Gln Leu
980 985 990

His Cys Pro Val Pro Lys Ala Gly Ala Ser Pro Val Asp Tyr Ser
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<400> 24

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Cys Ser Gly Ser Arg Leu Asp Tyr Lys Asp Asp Asp Asp Lys Arg Gln
            20                  25                  30

Glu Asp Phe Pro Pro Arg Ile Val Glu His Pro Ser Asp Leu Ile Val
        35                  40                  45

Ser Lys Gly Glu Pro Ala Thr Leu Asn Cys Lys Ala Glu Gly Arg Pro
    50                  55                  60

Thr Pro Thr Ile Glu Trp Tyr Lys Gly Gly Glu Arg Val Glu Thr Asp
65                  70                  75                  80

Lys Asp Asp Pro Arg Ser His Arg Met Leu Leu Pro Ser Gly Ser Leu
                85                  90                  95

Phe Phe Leu Arg Ile Val His Gly Arg Lys Ser Arg Pro Asp Glu Gly
            100                 105                 110

Val Tyr Val Cys Val Ala Arg Asn Tyr Leu Gly Glu Ala Val Ser His
        115                 120                 125

Asn Ala Ser Leu Glu Val Ala Ile Leu Arg Asp Asp Phe Arg Gln Asn
    130                 135                 140

Pro Ser Asp Val Met Val Ala Val Gly Glu Pro Ala Val Met Glu Cys
145                 150                 155                 160

Gln Pro Pro Arg Gly His Pro Glu Pro Thr Ile Ser Trp Lys Lys Asp
                165                 170                 175

Gly Ser Pro Leu Asp Asp Lys Asp Glu Arg Ile Thr Ile Arg Gly Gly
            180                 185                 190

Lys Leu Met Ile Thr Tyr Thr Arg Lys Ser Asp Ala Gly Lys Tyr Val
        195                 200                 205

Cys Val Gly Thr Asn Met Val Gly Glu Arg Glu Ser Glu Val Ala Glu
    210                 215                 220

Leu Thr Val Leu Glu Arg Pro Ser Phe Val Lys Arg Pro Ser Asn Leu
225                 230                 235                 240
```

```
Ala Val Thr Val Asp Asp Ser Ala Glu Phe Lys Cys Glu Ala Arg Gly
                245                 250                 255

Asp Pro Val Pro Thr Val Arg Trp Arg Lys Asp Asp Gly Glu Leu Pro
            260                 265                 270

Lys Ser Arg Tyr Glu Ile Arg Asp Asp His Thr Leu Lys Ile Arg Lys
        275                 280                 285

Val Thr Ala Gly Asp Met Gly Ser Tyr Thr Cys Val Ala Glu Asn Met
    290                 295                 300

Val Gly Lys Ala Glu Ala Ser Ala Thr Leu Thr Val Gln Val Gly Ser
305                 310                 315                 320

Glu Pro Pro His Phe Val Val Lys Pro Arg Asp Gln Val Val Ala Leu
                325                 330                 335

Gly Arg Thr Val Thr Phe Gln Cys Glu Ala Thr Gly Asn Pro Gln Pro
            340                 345                 350

Ala Ile Phe Trp Arg Arg Glu Gly Ser Gln Asn Leu Leu Phe Ser Tyr
        355                 360                 365

Gln Pro Pro Gln Ser Ser Ser Arg Phe Ser Val Ser Gln Thr Gly Asp
    370                 375                 380

Leu Thr Ile Thr Asn Val Gln Arg Ser Asp Val Gly Tyr Tyr Ile Cys
385                 390                 395                 400

Gln Thr Leu Asn Val Ala Gly Ser Ile Ile Thr Lys Ala Tyr Leu Glu
                405                 410                 415

Val Thr Asp Val Ile Ala Asp Arg Pro Pro Pro Val Ile Arg Gln Gly
            420                 425                 430

Pro Val Asn Gln Thr Val Ala Val Asp Gly Thr Phe Val Leu Ser Cys
        435                 440                 445

Val Ala Thr Gly Ser Pro Val Pro Thr Ile Leu Trp Arg Lys Asp Gly
    450                 455                 460

Val Leu Val Ser Thr Gln Asp Ser Arg Ile Lys Gln Leu Glu Asn Gly
465                 470                 475                 480

Val Leu Gln Ile Arg Tyr Ala Lys Leu Gly Asp Thr Gly Arg Tyr Thr
                485                 490                 495

Cys Ile Ala Ser Thr Pro Ser Gly Glu Ala Thr Trp Ser Ala Tyr Ile
            500                 505                 510
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```
Glu Val Gln Glu Phe Gly Val Pro Val Gln Pro Pro Arg Pro Thr Asp
        515                 520                 525

Pro Asn Leu Ile Pro Ser Ala Pro Ser Lys Pro Glu Val Thr Asp Val
    530                 535                 540

Ser Arg Asn Thr Val Thr Leu Ser Trp Gln Pro Asn Leu Asn Ser Gly
545                 550                 555                 560

Ala Thr Pro Thr Ser Tyr Ile Ile Glu Ala Phe Ser His Ala Ser Gly
                565                 570                 575

Ser Ser Trp Gln Thr Val Ala Glu Asn Val Lys Thr Glu Thr Ser Ala
            580                 585                 590

Ile Lys Gly Leu Lys Pro Asn Ala Ile Tyr Leu Phe Leu Val Arg Ala
        595                 600                 605

Ala Asn Ala Tyr Gly Ile Ser Asp Pro Ser Gln Ile Ser Asp Pro Val
    610                 615                 620

Lys Thr Gln Asp Val Leu Pro Thr Ser Gln Gly Val Asp His Lys Gln
625                 630                 635                 640

Val Gln Arg Glu Leu Gly Asn Ala Val Leu His Leu His Asn Pro Thr
                645                 650                 655

Val Leu Ser Ser Ser Ser Ile Glu Val His Trp Thr Val Asp Gln Gln
            660                 665                 670

Ser Gln Tyr Ile Gln Gly Tyr Lys Ile Leu Tyr Arg Pro Ser Gly Ala
        675                 680                 685

Asn His Gly Glu Ser Asp Trp Leu Val Phe Glu Val Arg Thr Pro Ala
    690                 695                 700

Lys Asn Ser Val Val Ile Pro Asp Leu Arg Lys Gly Val Asn Tyr Glu
705                 710                 715                 720

Ile Lys Ala Arg Pro Phe Phe Asn Glu Phe Gln Gly Ala Asp Ser Glu
                725                 730                 735

Ile Lys Phe Ala Lys Thr Leu Glu Glu Ala Pro Ser Ala Pro Pro Gln
            740                 745                 750

Gly Val Thr Val Ser Lys Asn Asp Gly Asn Gly Thr Ala Ile Leu Val
        755                 760                 765

Ser Trp Gln Pro Pro Pro Glu Asp Thr Gln Asn Gly Met Val Gln Glu
```

```
    770                 775                 780

Tyr Lys Val Trp Cys Leu Gly Asn Glu Thr Arg Tyr His Ile Asn Lys
785                 790                 795                 800

Thr Val Asp Gly Ser Thr Phe Ser Val Val Ile Pro Phe Leu Val Pro
                805                 810                 815

Gly Ile Arg Tyr Ser Val Glu Val Ala Ala Ser Thr Gly Ala Gly Ser
            820                 825                 830

Gly Val Lys Ser Glu Pro Gln Phe Ile Gln Leu Asp Ala His Gly Asn
        835                 840                 845

Pro Val Ser Pro Glu Asp Gln Val Ser Leu Ala Gln Gln Ile Ser Asp
    850                 855                 860

Val Val Lys Gln Pro Ala Phe Ile Ala Gly Ile Gly Ala Ala Cys Trp
865                 870                 875                 880

Ile Ile Leu Met Val Phe Ser Ile Trp Leu Tyr Arg His Arg Lys Lys
                885                 890                 895

Arg Asn Gly Leu Thr Ser Thr Tyr Ala Gly Ile Arg Lys Val Thr Tyr
            900                 905                 910

Gln Arg Gly Gly Glu Ala Val Ser Ser Gly Gly Arg Pro Gly Leu Leu
        915                 920                 925

Asn Ile Ser Glu Pro Ala Ala Gln Pro Trp Leu Ala Asp Thr Trp Pro
    930                 935                 940

Asn Thr Gly Asn Asn His Asn Asp Cys Ser Ile Ser Cys Cys Thr Ala
945                 950                 955                 960

Gly Asn Gly Asn Ser Asp Ser Asn Leu Thr Thr Tyr Ser Arg Pro Ala
                965                 970                 975

Asp Cys Ile Ala Asn Tyr Asn Asn Gln Leu Asp Asn Lys Gln Thr Asn
            980                 985                 990

Leu Met Leu Pro Glu Ser Thr Val  Tyr Gly Asp Val Asp  Leu Ser Asn
        995                 1000                1005

Lys Ile  Asn Glu Met Lys Thr  Phe Asn Ser Pro Asn  Leu Lys Asp
    1010                1015                1020

Gly Arg  Phe Val Asn Pro Ser  Gly Gln Pro Thr Pro  Tyr Ala Thr
    1025                1030                1035
```

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Thr Gln  Leu Ile Gln Ser Asn  Leu Ser Asn Asn Met  Asn Asn Gly
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Ser Gly  Asp Ser Gly Glu Lys  His Trp Lys Pro Leu  Gly Gln Gln
    1055                1060                1065

Lys Gln  Glu Val Ala Pro Val  Gln Tyr Asn Ile Val  Glu Gln Asn
    1070                1075                1080

Lys Leu  Asn Lys Asp Tyr Arg  Ala Asn Asp Thr Val  Pro Pro Thr
    1085                1090                1095

Ile Pro  Tyr Asn Gln Ser Tyr  Asp Gln Asn Thr Gly  Gly Ser Tyr
    1100                1105                1110

Asn Ser  Ser Asp Arg Gly Ser  Ser Thr Ser Gly Ser  Gln Gly His
    1115                1120                1125

Lys Lys  Gly Ala Arg Thr Pro  Lys Val Pro Lys Gln  Gly Gly Met
    1130                1135                1140

Asn Trp  Ala Asp Leu Leu Pro  Pro Pro Pro Ala His  Pro Pro Pro
    1145                1150                1155

His Ser  Asn Ser Glu Glu Tyr  Asn Ile Ser Val Asp  Glu Ser Tyr
    1160                1165                1170

Asp Gln  Glu Met Pro Cys Pro  Val Pro Pro Ala Arg  Met Tyr Leu
    1175                1180                1185

Gln Gln  Asp Glu Leu Glu Glu  Glu Glu Asp Glu Arg  Gly Pro Thr
    1190                1195                1200

Pro Pro  Val Arg Gly Ala Ala  Ser Ser Pro Ala Ala  Val Ser Tyr
    1205                1210                1215

Ser His  Gln Ser Thr Ala Thr  Leu Thr Pro Ser Pro  Gln Glu Glu
    1220                1225                1230

Leu Gln  Pro Met Leu Gln Asp  Cys Pro Glu Glu Thr  Gly His Met
    1235                1240                1245

Gln His  Gln Pro Asp Arg Arg  Arg Gln Pro Val Ser  Pro Pro Pro
    1250                1255                1260

Pro Pro  Arg Pro Ile Ser Pro  Pro His Thr Tyr Gly  Tyr Ile Ser
    1265                1270                1275

Gly Pro  Leu Val Ser Asp Met  Asp Thr Asp Ala Pro  Glu Glu Glu
    1280                1285                1290
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Glu Asp  Glu Ala Asp Met Glu  Val Ala Lys Met Gln  Thr Arg Arg
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Leu Leu  Leu Arg Gly Leu Glu  Gln Thr Pro Ala Ser  Ser Val Gly
    1310                1315                1320

Asp Leu  Glu Ser Ser Val Thr  Gly Ser Met Ile Asn  Gly Trp Gly
    1325                1330                1335

Ser Ala  Ser Glu Glu Asp Asn  Ile Ser Ser Gly Arg  Ser Ser Val
    1340                1345                1350

Ser Ser  Ser Asp Gly Ser Phe  Phe Thr Asp Ala Asp  Phe Ala Gln
    1355                1360                1365

Ala Val  Ala Ala Ala Ala Glu  Tyr Ala Gly Leu Lys  Val Ala Arg
    1370                1375                1380

Arg Gln  Met Gln Asp Ala Ala  Gly Arg Arg His Phe  His Ala Ser
    1385                1390                1395

Gln Cys  Pro Arg Pro Thr Ser  Pro Val Ser Thr Asp  Ser Asn Met
    1400                1405                1410

Ser Ala  Ala Val Met Gln Lys  Thr Arg Pro Ala Lys  Lys Leu Lys
    1415                1420                1425

His Gln  Pro Gly His Leu Arg  Arg Glu Thr Tyr Thr  Asp Asp Leu
    1430                1435                1440

Pro Pro  Pro Pro Val Pro Pro  Pro Ala Ile Lys Ser  Pro Thr Ala
    1445                1450                1455

Gln Ser  Lys Thr Gln Leu Glu  Val Arg Pro Val Val  Val Pro Lys
    1460                1465                1470

Leu Pro  Ser Met Asp Ala Arg  Thr Asp Arg Ser Ser  Asp Arg Lys
    1475                1480                1485

Gly Ser  Ser Tyr Lys Gly Arg  Glu Val Leu Asp Gly  Arg Gln Val
    1490                1495                1500

Val Asp  Met Arg Thr Asn Pro  Gly Asp Pro Arg Glu  Ala Gln Glu
    1505                1510                1515

Gln Gln  Asn Asp Gly Lys Gly  Arg Gly Asn Lys Ala  Ala Lys Arg
    1520                1525                1530

Asp Leu  Pro Pro Ala Lys Thr  His Leu Ile Gln Glu  Asp Ile Leu
    1535                1540                1545
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Pro Tyr  Cys Arg Pro Thr Phe  Pro Thr Ser Asn Asn  Pro Arg Asp
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Pro Ser  Ser Ser Ser Ser Met  Ser Ser Arg Gly Ser  Gly Ser Arg
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Gln Arg  Glu Gln Ala Asn Val  Gly Arg Arg Asn Ile  Ala Glu Met
    1580                1585                1590

Gln Val  Leu Gly Gly Tyr Glu  Arg Gly Glu Asp Asn  Asn Glu Glu
    1595                1600                1605

Leu Glu  Glu Thr Glu Ser
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Tyr Lys Asp Gly Glu Arg Val Glu Thr Asp Lys Asp Asp Pro Arg Ser
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His Arg Met Leu Leu Pro Ser Gly Ser Leu Phe Phe Leu Arg Ile Val
                85                  90                  95

His Gly Arg Arg Ser Lys Pro Asp Glu Gly Ser Tyr Val Cys Val Ala
            100                 105                 110

Arg Asn Tyr Leu Gly Glu Ala Val Ser Arg Asn Ala Ser Leu Glu Val
        115                 120                 125

Ala Leu Leu Arg Asp Asp Phe Arg Gln Asn Pro Thr Asp Val Val Val
    130                 135                 140

Ala Ala Gly Glu Pro Ala Ile Leu Glu Cys Gln Pro Pro Arg Gly His
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Pro Glu Pro Thr Ile Tyr Trp Lys Lys Asp Lys Val Arg Ile Asp Asp
                165                 170                 175

Lys Glu Glu Arg Ile Ser Ile Arg Gly Gly Lys Leu Met Ile Ser Asn
            180                 185                 190

Thr Arg Lys Ser Asp Ala Gly Met Tyr Thr Cys Val Gly Thr Asn Met
        195                 200                 205

Val Gly Glu Arg Asp Ser Asp Pro Ala Glu Leu Thr Val Phe Glu Arg
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Pro Thr Phe Leu Arg Arg Pro Ile Asn Gln Val Val Leu Glu Glu Glu
225                 230                 235                 240

Ala Val Glu Phe Arg Cys Gln Val Gln Gly Asp Pro Gln Pro Thr Val
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Arg Trp Lys Lys Asp Asp Ala Asp Leu Pro Arg Gly Arg Tyr Asp Ile
            260                 265                 270

Lys Asp Asp Tyr Thr Leu Arg Ile Lys Lys Thr Met Ser Thr Asp Glu
        275                 280                 285

Gly Thr Tyr Met Cys Ile Ala Glu Asn Arg Val Gly Lys Met Glu Ala
    290                 295                 300

Ser Ala Thr Leu Thr Val Arg Ala Arg Pro Val Ala Pro Pro Gln Phe
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Phe Pro Cys Glu Thr Lys Gly Asn Pro Gln Pro Ala Val Phe Trp Gln
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Lys Glu Gly Ser Gln Asn Leu Leu Phe Pro Asn Gln Pro Gln Gln Pro
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Ile Gln Arg Ser Asp Ala Gly Tyr Tyr Ile Cys Gln Ala Leu Thr Val
385                 390                 395                 400

Ala Gly Ser Ile Leu Ala Lys Ala Gln Leu Glu Val Thr Asp Val Leu
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Thr Asp Arg Pro Pro Pro Ile Ile Leu Gln Gly Pro Ala Asn Gln Thr
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Leu Ala Val Asp Gly Thr Ala Leu Leu Lys Cys Lys Ala Thr Gly Asp
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Pro Leu Pro Val Ile Ser Trp Leu Lys Glu Gly Phe Thr Phe Pro Gly
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Arg Asp Pro Arg Ala Thr Ile Gln Glu Gln Gly Thr Leu Gln Ile Lys
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Asn Leu Arg Ile Ser Asp Thr Gly Thr Tyr Thr Cys Val Ala Thr Ser
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Ser Ser Gly Glu Thr Ser Trp Ser Ala Val Leu Asp Val Thr Glu Ser
            500                 505                 510

Gly Ala Thr Ile Ser Lys Asn Tyr Asp Leu Ser Asp Leu Pro Gly Pro
        515                 520                 525

Pro Ser Lys Pro Gln Val Thr Asp Val Thr Lys Asn Ser Val Thr Leu
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Ser Trp Gln Pro Gly Thr Pro Gly Thr Leu Pro Ala Ser Ala Tyr Ile
545                 550                 555                 560

Ile Glu Ala Phe Ser Gln Ser Val Ser Asn Ser Trp Gln Thr Val Ala
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Asn His Val Lys Thr Thr Leu Tyr Thr Val Arg Gly Leu Arg Pro Asn
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Thr Ile Tyr Leu Phe Met Val Arg Ala Ile Asn Pro Gln Gly Leu Ser
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Asp Pro Ser Pro Met Ser Asp Pro Val Arg Thr Gln Asp Ile Ser Pro
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Pro Ala Gln Gly Val Asp His Arg Gln Val Gln Lys Glu Leu Gly Asp
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Val Leu Val Arg Leu His Asn Pro Val Val Leu Thr Pro Thr Thr Val
                645                 650                 655

Gln Val Thr Trp Thr Val Asp Arg Gln Pro Gln Phe Ile Gln Gly Tyr
            660                 665                 670

Arg Val Met Tyr Arg Gln Thr Ser Gly Leu Gln Ala Thr Ser Ser Trp
        675                 680                 685

Gln Asn Leu Asp Ala Lys Val Pro Thr Glu Arg Ser Ala Val Leu Val
    690                 695                 700

Asn Leu Lys Lys Gly Val Thr Tyr Glu Ile Lys Val Arg Pro Tyr Phe
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Asn Glu Phe Gln Gly Met Asp Ser Glu Ser Lys Thr Val Arg Thr Thr
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Glu Glu Ala Pro Ser Ala Pro Pro Gln Ser Val Thr Val Leu Thr Val
            740                 745                 750

Gly Ser Tyr Asn Ser Thr Ser Ile Ser Val Ser Trp Asp Pro Pro Pro
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Pro Asp His Gln Asn Gly Ile Ile Gln Glu Tyr Lys Ile Trp Cys Leu
    770                 775                 780

Gly Asn Glu Thr Arg Phe His Ile Asn Lys Thr Val Asp Ala Ala Ile
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Arg Ser Val Ile Ile Gly Gly Leu Phe Pro Gly Ile Gln Tyr Arg Val
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Glu Val Ala Ala Ser Thr Ser Ala Gly Val Gly Val Lys Ser Glu Pro
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Gln Pro Ile Ile Ile Gly Arg Arg Asn Glu Val Val Ile Thr Glu Asn
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Asn Asn Ser Ile Thr Glu Gln Ile Thr Asp Val Val Lys Gln Pro Ala
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Phe Ile Ala Gly Ile Gly Gly Ala Cys Trp Val Ile Leu Met Gly Phe
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Ser Ile Trp Leu Tyr Trp Arg Arg Lys Lys Arg Lys Gly Leu Ser Asn
                885                 890                 895

Tyr Ala Val Thr Phe Gln Arg Gly Asp Gly Gly Leu Met Ser Asn Gly
            900                 905                 910

Ser Arg Pro Gly Leu Leu Asn Ala Gly Asp Pro Ser Tyr Pro Trp Leu
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Ala Asp Ser Trp Pro Ala Thr Ser Leu Pro Val Asn Asn Ser Asn Ser
    930                 935                 940

Gly Pro Asn Glu Ile Gly Asn Phe Gly Arg Gly Asp Val Leu Pro Pro
945                 950                 955                 960

Val Pro Gly Gln Gly Asp Lys Thr Ala Thr Met Leu Ser Asp Gly Ala
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Ile Tyr Ser Ser Ile Asp Phe Thr Thr Lys Thr Ser Tyr Asn Ser Ser
            980                 985                 990

Ser Gln Ile Thr Gln Ala Thr Pro  Tyr Ala Thr Thr Gln  Ile Leu His
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Ser Asn  Ser Ile His Glu Leu  Ala Val Asp Leu Pro  Asp Pro Gln
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Trp Lys  Ser Ser Ile Gln Gln  Lys Thr Asp Leu Met  Gly Phe Gly
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Tyr Ser  Leu Pro Asp Gln Asn  Lys Gly Asn Asn Gly  Gly Lys Gly
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Gly Lys  Lys Lys Lys Asn Lys  Asn Ser Ser Lys Pro  Gln Lys Asn
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Asn Gly  Ser Thr Trp Ala Asn  Val Pro Leu Pro Pro  Pro Pro Val
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Gln Pro  Leu Pro Gly Thr Glu  Leu Glu His Tyr Ala  Val Glu Gln
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Gln Glu  Asn Gly Tyr Asp Ser  Asp Ser Trp Cys Pro  Pro Leu Pro
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Val Gln  Thr Tyr Leu His Gln  Gly Leu Glu Asp Glu  Leu Glu Glu
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Asp Asp  Asp Arg Val Pro Thr  Pro Pro Val Arg Gly  Val Ala Ser
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Ser Pro  Ala Ile Ser Phe Gly  Gln Gln Ser Thr Ala  Thr Leu Thr
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Pro Ser  Pro Arg Glu Glu Met  Gln Pro Met Leu Gln  Ala His Leu
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Asp Glu  Leu Thr Arg Ala Tyr  Gln Phe Asp Ile Ala  Lys Gln Thr
    1175                1180                1185

Trp His  Ile Gln Ser Asn Asn  Gln Pro Pro Gln Pro  Pro Val Pro
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Pro Leu  Gly Tyr Val Ser Gly  Ala Leu Ile Ser Asp  Leu Glu Thr
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Asp Val  Ala Asp Asp Asp Ala  Asp Asp Glu Glu Glu  Ala Leu Glu
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Ile Pro  Arg Pro Leu Arg Ala  Leu Asp Gln Thr Pro  Gly Ser Ser
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Met Asp  Asn Leu Asp Ser Ser  Val Thr Gly Ser Met  Val Asn Gly
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Trp Gly  Ser Ala Ser Asp Glu  Asp Arg Asn Phe Ser  Ser His Arg
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Ser Ser  Val Gly Ser Ser Ser  Asp Gly Ser Ile Phe  Ala Ser Gly
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Ser Phe  Ala Gln Ala Leu Val  Ala Ala Ala Asp Lys  Ala Gly Phe
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Arg Leu  Asp Gly Thr Ser Leu  Thr Arg Thr Gly Lys  Ala Phe Thr
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Ser Ser  Gln Arg Pro Arg Pro  Thr Ser Pro Phe Ser  Thr Asp Ser
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Asn Thr  Ser Ala Ala Leu Ser  Gln Ser Gln Arg Pro  Arg Pro Thr
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Lys Lys  His Lys Gly Gly Arg  Met Asp Gln Gln Pro  Ala Leu Pro
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His Arg  Arg Glu Gly Met Thr  Asp Asp Leu Pro Pro  Pro Pro Asp
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Pro Pro  Pro Gly Gln Gly Leu  Arg Gln Gln Ile Gly  Pro Ser Gln
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Gln Ala  Gly Asn Val Glu Asn  Ser Ala Glu Arg Lys  Gly Ser Ser
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Leu Glu  Arg Gln His Ala Ser  Ser Leu Glu Asp Thr  Lys Ser Ser
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Leu Asp  Cys Pro Ala Arg Thr  Ser Leu Glu Trp Gln  Arg Gln Thr
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Gln Glu  Trp Ile Ser Ser Thr  Glu Arg Gln Glu Asp  Ile Arg Lys
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Ala Pro  His Lys Gln Gly Val  Gly Ser Glu Glu Ala  Leu Val Pro
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Tyr Ser  Lys Pro Ser Phe Pro  Ser Pro Gly Gly His  Ser Ser Ser
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Gly Thr  Ala Ser Ser Lys Gly  Ser Thr Gly Pro Arg  Lys Thr Glu
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Val Leu  Arg Ala Gly His Gln  Arg Asn Ala Ser Asp  Leu Leu Asp
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ccacttcaag gaccccgtgc tcgattccgg aagaaaccca aggctcttcc ctacaggagg 3780

gagaacagtc ctggggactt gcccccacca cccttgccac cgccagagga agaggcgagc 3840

tgggccctag agctgagggc agcaggcagc atgtcctccc tggagcggga gcgcagtggg 3900

gagaggaaag cggtccaggc cgtgcccctg gcagcccagc gggtgctcca cccagatgaa 3960

gaggcctggc tcccatacag cagaccaagc ttcctgtccc ggggccaggg caccagcaca 4020

tgttccacgg ccggcagcaa ctcttccagg ggctccagca gctctagggg ctcccggggc 4080

cctggccgga gccggagtcg gagtcagagc cggagccaga gccaaaggcc aggacagaaa 4140

cgccgagagg aaccaagatg a 4161

<210> 28
<211> 1386
<212> PRT
<213> Homo sapiens

<400> 28

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Met Leu Arg Tyr Leu Leu Lys Thr Leu Leu Gln Met Asn Leu Phe Ala
1               5                  10                  15

Asp Ser Leu Ala Gly Asp Ile Ser Asn Ser Ser Glu Leu Leu Leu Gly
            20                  25                  30

Phe Asn Ser Ser Leu Ala Ala Leu Asn His Thr Leu Leu Pro Pro Gly
        35                  40                  45

Asp Pro Ser Leu Asn Gly Ser Arg Val Gly Pro Glu Asp Ala Met Pro
    50                  55                  60

Arg Ile Val Glu Gln Pro Pro Asp Leu Leu Val Ser Arg Gly Glu Pro
65                  70                  75                  80

Ala Thr Leu Pro Cys Arg Ala Glu Gly Arg Pro Arg Pro Asn Ile Glu
                85                  90                  95

Trp Tyr Lys Asn Gly Ala Arg Val Ala Thr Val Arg Glu Asp Pro Arg
            100                 105                 110

Ala His Arg Leu Leu Leu Pro Ser Gly Ala Leu Phe Phe Pro Arg Ile
        115                 120                 125

Val His Gly Arg Arg Ala Arg Pro Asp Glu Gly Val Tyr Thr Cys Val
    130                 135                 140

Ala Arg Asn Tyr Leu Gly Ala Ala Ala Ser Arg Asn Ala Ser Leu Glu
145                 150                 155                 160

Val Ala Val Leu Arg Asp Asp Phe Arg Gln Ser Pro Gly Asn Val Val
                165                 170                 175

Val Ala Val Gly Glu Pro Ala Val Leu Glu Cys Val Pro Pro Arg Gly
            180                 185                 190

His Pro Glu Pro Ser Val Ser Trp Arg Lys Asp Gly Ala Arg Leu Lys
        195                 200                 205

Glu Glu Glu Gly Arg Ile Thr Ile Arg Gly Gly Lys Leu Met Met Ser
    210                 215                 220

His Thr Leu Lys Ser Asp Ala Gly Met Tyr Val Cys Val Ala Ser Asn
225                 230                 235                 240

Met Ala Gly Glu Arg Glu Ser Ala Ala Ala Glu Val Met Val Leu Glu
                245                 250                 255
```

```
Arg Pro Ser Phe Leu Arg Arg Pro Val Asn Gln Val Val Leu Ala Asp
            260                 265                 270

Ala Pro Val Thr Phe Leu Cys Glu Val Lys Gly Asp Pro Pro Pro Arg
        275                 280                 285

Leu Arg Trp Arg Lys Glu Asp Gly Glu Leu Pro Thr Gly Arg Tyr Glu
    290                 295                 300

Ile Arg Ser Asp His Ser Leu Trp Ile Gly His Val Ser Ala Glu Asp
305                 310                 315                 320

Glu Gly Thr Tyr Thr Cys Val Ala Glu Asn Ser Val Gly Arg Ala Glu
                325                 330                 335

Ala Ser Gly Ser Leu Ser Val His Val Pro Pro Gln Leu Val Thr Gln
            340                 345                 350

Pro Gln Asp Gln Met Ala Ala Pro Gly Glu Ser Val Ala Phe Gln Cys
        355                 360                 365

Glu Thr Lys Gly Asn Pro Pro Pro Ala Ile Phe Trp Gln Lys Glu Gly
    370                 375                 380

Ser Gln Val Leu Leu Phe Pro Ser Gln Ser Leu Gln Pro Thr Gly Arg
385                 390                 395                 400

Phe Ser Val Ser Pro Arg Gly Gln Leu Asn Ile Thr Ala Val Gln Arg
                405                 410                 415

Gly Asp Ala Gly Tyr Tyr Val Cys Gln Ala Val Ser Val Ala Gly Ser
            420                 425                 430

Ile Leu Ala Lys Ala Leu Leu Glu Ile Lys Gly Ala Ser Leu Asp Gly
        435                 440                 445

Leu Pro Pro Val Ile Leu Gln Gly Pro Ala Asn Gln Thr Leu Val Leu
    450                 455                 460

Gly Ser Ser Val Trp Leu Pro Cys Arg Val Thr Gly Asn Pro Gln Pro
465                 470                 475                 480

Ser Val Arg Trp Lys Lys Asp Gly Gln Trp Leu Gln Gly Asp Asp Leu
                485                 490                 495

Gln Phe Lys Thr Met Ala Asn Gly Thr Leu Tyr Ile Ala Asn Val Gln
            500                 505                 510

Glu Met Asp Met Gly Phe Tyr Ser Cys Val Ala Lys Ser Ser Thr Gly
        515                 520                 525
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Glu Ala Thr Trp Ser Gly Trp Leu Lys Met Arg Glu Asp Trp Gly Val
    530                 535                 540

Ser Pro Asp Pro Pro Thr Glu Pro Ser Ser Pro Pro Gly Ala Pro Ser
545                 550                 555                 560

Gln Pro Val Val Thr Glu Ile Thr Lys Asn Ser Ile Thr Leu Thr Trp
                565                 570                 575

Lys Pro Asn Pro Gln Thr Gly Ala Ala Val Thr Ser Tyr Val Ile Glu
            580                 585                 590

Ala Phe Ser Pro Ala Ala Gly Asn Thr Trp Arg Thr Val Ala Asp Gly
        595                 600                 605

Val Gln Leu Glu Thr His Thr Val Ser Gly Leu Gln Pro Asn Thr Ile
    610                 615                 620

Tyr Leu Phe Leu Val Arg Ala Val Gly Ala Trp Gly Leu Ser Glu Pro
625                 630                 635                 640

Ser Pro Val Ser Glu Pro Val Arg Thr Gln Asp Ser Ser Pro Ser Arg
                645                 650                 655

Pro Val Glu Asp Pro Trp Arg Gly Gln Gln Gly Leu Ala Glu Val Ala
            660                 665                 670

Val Arg Leu Gln Glu Pro Ile Val Leu Gly Pro Arg Thr Leu Gln Val
        675                 680                 685

Ser Trp Thr Val Asp Gly Pro Val Gln Leu Val Gln Gly Phe Arg Val
    690                 695                 700

Ser Trp Arg Val Ala Gly Pro Glu Gly Gly Ser Trp Thr Met Leu Asp
705                 710                 715                 720

Leu Gln Ser Pro Ser Gln Gln Ser Thr Val Leu Arg Gly Leu Pro Pro
                725                 730                 735

Gly Thr Gln Ile Gln Ile Lys Val Gln Ala Gln Gly Gln Glu Gly Leu
            740                 745                 750

Gly Ala Glu Ser Leu Ser Val Thr Arg Ser Ile Pro Glu Glu Ala Pro
        755                 760                 765

Ser Gly Pro Pro Gln Gly Val Ala Val Ala Leu Gly Gly Asp Gly Asn
    770                 775                 780

Ser Ser Ile Thr Val Ser Trp Glu Pro Pro Leu Pro Ser Gln Gln Asn
785                 790                 795                 800
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```
Gly Val Ile Thr Glu Tyr Gln Ile Trp Cys Leu Gly Asn Glu Ser Arg
                805                 810                 815

Phe His Leu Asn Arg Ser Ala Ala Gly Trp Ala Arg Ser Ala Met Leu
            820                 825                 830

Arg Gly Leu Val Pro Gly Leu Leu Tyr Arg Thr Leu Val Ala Ala Ala
        835                 840                 845

Thr Ser Ala Gly Val Gly Val Pro Ser Ala Pro Val Leu Val Gln Leu
    850                 855                 860

Pro Ser Pro Pro Asp Leu Glu Pro Gly Leu Glu Val Gly Ala Gly Leu
865                 870                 875                 880

Ala Val Arg Leu Ala Arg Val Leu Arg Glu Pro Ala Phe Leu Ala Gly
                885                 890                 895

Ser Gly Ala Ala Cys Gly Ala Leu Leu Leu Gly Leu Cys Ala Ala Leu
            900                 905                 910

Tyr Trp Arg Arg Lys Gln Arg Lys Glu Leu Ser His Tyr Thr Ala Ser
        915                 920                 925

Phe Ala Tyr Thr Pro Ala Val Ser Phe Pro His Ser Glu Gly Leu Ser
    930                 935                 940

Gly Ala Ser Ser Arg Pro Pro Met Gly Leu Gly Pro Ala Pro Tyr Ser
945                 950                 955                 960

Trp Leu Ala Asp Ser Trp Pro His Pro Ser Arg Ser Pro Ser Ala Gln
                965                 970                 975

Glu Pro Arg Gly Ser Cys Cys Pro Ser Asn Pro Asp Pro Asp Asp Arg
            980                 985                 990

Tyr Tyr Asn Glu Ala Gly Ile Ser  Leu Tyr Leu Ala Gln  Thr Ala Arg
        995                 1000                1005

Gly Thr  Ala Ala Pro Gly Glu  Gly Pro Val Tyr Ser  Thr Ile Asp
    1010                1015                1020

Pro Ala  Gly Glu Glu Leu Gln  Thr Phe His Gly Gly  Phe Pro Gln
    1025                1030                1035

His Pro  Ser Gly Asp Leu Gly  Pro Trp Ser Gln Tyr  Ala Pro Pro
    1040                1045                1050

Glu Trp  Ser Gln Gly Asp Ser  Gly Ala Lys Gly Gly  Lys Val Lys
```

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    1055                1060                1065

Leu Leu  Gly Lys Pro Val Gln  Met Pro Ser Leu Asn  Trp Pro Glu
    1070                1075                1080

Ala Leu  Pro Pro Pro Pro Pro  Ser Cys Glu Leu Ser  Cys Leu Glu
    1085                1090                1095

Gly Pro  Glu Glu Glu Leu Glu  Gly Ser Ser Glu Pro  Glu Glu Trp
    1100                1105                1110

Cys Pro  Pro Met Pro Glu Arg  Ser His Leu Thr Glu  Pro Ser Ser
    1115                1120                1125

Ser Gly  Gly Cys Leu Val Thr  Pro Ser Arg Arg Glu  Thr Pro Ser
    1130                1135                1140

Pro Thr  Pro Ser Tyr Gly Gln  Gln Ser Thr Ala Thr  Leu Thr Pro
    1145                1150                1155

Ser Pro  Pro Asp Pro Pro Gln  Pro Pro Thr Asp Met  Pro His Leu
    1160                1165                1170

His Gln  Met Pro Arg Arg Val  Pro Leu Gly Pro Ser  Ser Pro Leu
    1175                1180                1185

Ser Val  Ser Gln Pro Met Leu  Gly Ile Arg Glu Ala  Arg Pro Ala
    1190                1195                1200

Gly Leu  Gly Ala Gly Pro Ala  Ala Ser Pro His Leu  Ser Pro Ser
    1205                1210                1215

Pro Ala  Pro Ser Thr Ala Ser  Ser Ala Pro Gly Arg  Thr Trp Gln
    1220                1225                1230

Gly Asn  Gly Glu Met Thr Pro  Pro Leu Gln Gly Pro  Arg Ala Arg
    1235                1240                1245

Phe Arg  Lys Lys Pro Lys Ala  Leu Pro Tyr Arg Arg  Glu Asn Ser
    1250                1255                1260

Pro Gly  Asp Leu Pro Pro Pro  Pro Leu Pro Pro Pro  Glu Glu Glu
    1265                1270                1275

Ala Ser  Trp Ala Leu Glu Leu  Arg Ala Ala Gly Ser  Met Ser Ser
    1280                1285                1290

Leu Glu  Arg Glu Arg Ser Gly  Glu Arg Lys Ala Val  Gln Ala Val
    1295                1300                1305
```

Pro Leu Ala Ala Gln Arg Val Leu His Pro Asp Glu Glu Ala Trp
1310 1315 1320

Leu Pro Tyr Ser Arg Pro Ser Phe Leu Ser Arg Gly Gln Gly Thr
1325 1330 1335

Ser Thr Cys Ser Thr Ala Gly Ser Asn Ser Ser Arg Gly Ser Ser
1340 1345 1350

Ser Ser Arg Gly Ser Arg Gly Pro Gly Arg Ser Arg Ser Arg Ser
1355 1360 1365

Gln Ser Arg Ser Gln Ser Gln Arg Pro Gly Gln Lys Arg Arg Glu
1370 1375 1380

Glu Pro Arg
1385

<210> 29
<211> 195
<212> DNA
<213> Artificial Sequence

<220>
<223> IL-8 promoter region

<400> 29
gataaggaac aaataggaag tgtgatgact caggtttgcc ctgaggggat gggccatcag 60

ttgcaaatcg tggaatttcc tctgacataa tgaaaagatg agggtgcata agttctctag 120

tagggtgatg atataaaaag ccaccggagc actccataag gcacaaactt tcagagacag 180

cagagcacac aagct 195

<210> 30
<211> 354
<212> DNA
<213> Mus musculus

<400> 30
gaagtgcagc tggtggagtc tgggggaggc ttagtgaagc ctggagggtc cctgaaactc 60

tcctgtgcag cctctggatt cactttcagt acctatgcca tgtcttgggt tcgccagact 120

ccggagaaga ggctggagtg ggtcgcaacc attagtaatg gtggtactta cacctactat 180

ccagacagtg tgaagggtcg attcaccatc tccagagaca atgccgagaa caccctgtac 240

ctgcaaatga gcagtctgag gtctgaggac acggccatgt atttctgtgc aagactaatc 300

tactatgatt accttgacta ctggggccac ggcaccactc tcacagtctc ctca 354

<210> 31
<211> 118
<212> PRT
<213> Mus musculus

<400> 31

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Thr Ile Ser Asn Gly Gly Thr Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Glu Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Phe Cys
85 90 95

Ala Arg Leu Ile Tyr Tyr Asp Tyr Leu Asp Tyr Trp Gly His Gly Thr
100 105 110

Thr Leu Thr Val Ser Ser
115

<210> 32
<211> 342
<212> DNA
<213> Mus musculus

<400> 32
gatgctgtga tgacccaaac tccactctcc ctgcctgtca gtcttggaga tcaagcctcc 60

atctcttgca ggtctagtca gagccttgaa aacagtaatg gaaacaccta tttgaactgg 120

tacttccaga aaccaggcca gtctccacag ctcctgatct acagggtttc caaccgattt 180

tctggggtcc tagacaggtt cagtggtagt ggatcaggga cagatttcac actgaaaatc 240

agcagagtgg aggctgaaga tttcggagtt tatttctgcc tccaagttac tcatgtcccg 300

tggacgttcg gtggaggcac caagctggaa atcaaacggg ct 342

<210> 33
<211> 114
<212> PRT
<213> Mus musculus

<400> 33

Asp Ala Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Glu Asn Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Asn Trp Tyr Phe Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser Gly Val Leu
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Phe Gly Val Tyr Phe Cys Leu Gln Val
85 90 95

Thr His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg Ala

<210> 34
<211> 449
<212> DNA
<213> Homo sapiens

<400> 34
gcctccggac tctagagcca ccatggtgct gcagacccag gtgttcatct ccctgctgct 60

gtggatctcc ggcgcgtacg gcgatatcgt gatgattaaa cgtacggtgg ccgccccctc 120

cgtgttcatc ttcccccccct ccgacgagca gctgaagtcc ggcaccgcct ccgtggtgtg 180

cctgctgaat aacttctacc ccagagaggc caaggtgcag tggaaggtgg acaacgccct 240

gcagtccggg aactcccagg agagcgtgac cgagcaggac agcaaggaca gcacctacag 300

cctgagcagc accctgaccc tgagcaaagc cgactacgag aagcacaagg tgtacgcctg 360

cgaggtgacc caccagggcc tgagctcccc cgtcaccaag agcttcaaca gggggggagtg 420

ttaggggccc gtttaaacgg gggaggcta 449

<210> 35
<211> 1132
<212> DNA
<213> Artificial Sequence

<220>
<223> human H-chain signal and IgG1 constant DNA

<400> 35
gcctccggac tctagagcca ccatgaaaca cctgtggttc ttcctcctgc tggtggcagc 60

tcccagatgg gtgctgagcc aggtgcaatt gtgcaggcgg ttagctcagc ctccaccaag 120

ggcccaagcg tcttcccccct ggcaccctcc tccaagagca cctctggcgg cacagccgcc 180

ctgggctgcc tggtcaagga ctacttcccc gaacccgtga ccgtgagctg gaactcaggc 240

gccctgacca gcggcgtgca caccttcccc gctgtcctgc agtcctcagg actctactcc 300

ctcagcagcg tggtgaccgt gccctccagc agcttgggca cccagaccta catctgcaac 360

gtgaatcaca agcccagcaa caccaaggtg gacaagagag ttgagcccaa atcttgtgac 420

aaaactcaca catgcccacc ctgcccagca cctgaactcc tgggggggacc ctcagtcttc 480

ctcttccccc caaaacccaa ggacaccctc atgatctccc ggacccctga ggtcacatgc 540

gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc 600

gtggaggtgc ataatgccaa gacaaagccc cgggaggagc agtacaacag cacgtaccgg 660

gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc 720

aaggtctcca acaaagccct cccagccccc atcgagaaaa ccatctccaa agccaaaggc 780

cagccccggg aaccacaggt gtacaccctg cccccatccc gggaggagat gaccaagaac 840

caggtcagcc tgacctgcct ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg 900

gagagcaatg gccagcccga gaacaactac aagaccaccc ctcccgtgct ggactccgac 960

ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcagggcaac 1020

gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacaccca gaagagcctc 1080

tccctgtctc ccggcaaatg agatatcggg cccgtttaaa cgggggaggc ta 1132

<210> 36
<211> 1118
<212> DNA
<213> Homo sapiens

<400> 36
gcctccggac tctagagcca ccatgaaaca cctgtggttc ttcctcctgc tggtggcagc 60

tcccagatgg gtgctgagcc aggtgcaatt gtgcaggcgg ttagctcagc cagcaccaag 120

ggcccttccg tgttccctct ggccccttgt agccgttcca ccagcgagtc caccgccgcc 180

cttggctgtc tggtgaagga ctacttccct gagcctgtga ccgtgagctg gaactccgga 240

gcccttacca gcggcgtgca caccttccct gccgtgctgc agtccagcgg cctttactcc 300

ctgagctccg tggtgaccgt gcctagctcc aacttcggca cccaaaccta cacctgtaac 360

gtggaccaca agcctagcaa caccaaggtg gacaagaccg tggagcgtaa gtgttgtgtg 420

gagtgtcctc cttgtcctgc ccctcctgtg gccggacctt ccgtgttcct tttccctcct 480

aagcctaagg acaccctgat gatcagccgt acccctgagg tgacctgtgt ggtggtggac 540

gtgtcccacg aggaccctga ggtgcagttc aactggtacg tggacggcgt ggaggtgcac 600

aacgccaaga ccaagcctcg tgaggagcaa ttcaacagca ccttccgtgt ggtgtccgtg 660

cttaccgtgg tgcaccaaga ctggctgaac ggcaaggagt acaagtgtaa ggtgagcaac 720

aagggacttc ctgcccctat cgagaagacc atctccaaga ccaagggcca acctcgtgag 780

cctcaagtgt acacccttcc tcctagccgt gaggagatga ccaagaacca agtgtccctt 840

acctgtctgg tgaagggctt ctaccctagc gacatcgccg tggagtggga gtccaacgga 900

caacctgaga acaactacaa gaccacccct cctatgcttg acagcgacgg ctccttcttc 960

ctgtacagca agctgaccgt ggacaagtcc cgttggcaac aaggcaacgt gttcagctgt 1020

tccgtgatgc acgaggccct gcacaaccac tacacccaaa agagcctttc cctgagccct 1080

ggaaagtgat atcgggcccg tttaaacggg ggaggcta 1118

<210> 37
<211> 717
<212> DNA
<213> Artificial Sequence

<220>
<223> cMAb1 L chain na

<400> 37
atggtgctgc agacccaggt gttcatctcc ctgctgctgt ggatctccgg cgcgtacggc 60

gatgctgtga tgacccaaac tccactctcc ctgcctgtca gtcttggaga tcaagcctcc 120

atctcttgca ggtctagtca gagccttgaa aacagtaatg gaaacaccta tttgaactgg 180

tacttccaga aaccaggcca gtctccacag ctcctgatct acagggtttc caaccgattt 240

tctggggtcc tagacaggtt cagtggtagt ggatcaggga cagatttcac actgaaaatc 300

agcagagtgg aggctgaaga tttcggagtt tatttctgcc tccaagttac tcatgtcccg 360

tggacgttcg gtggaggcac caagctggaa atcaaacggg ctgtggccgc cccctccgtg 420

ttcatcttcc ccccctccga cgagcagctg aagtccggca ccgcctccgt ggtgtgcctg 480

ctgaataact tctaccccag agaggccaag gtgcagtgga aggtggacaa cgccctgcag 540

tccgggaact cccaggagag cgtgaccgag caggacagca aggacagcac ctacagcctg 600

agcagcaccc tgaccctgag caaagccgac tacgagaagc acaaggtgta cgcctgcgag 660

gtgacccacc agggcctgag ctcccccgtc accaagagct tcaacagggg ggagtgt 717

<210> 38
<211> 239
<212> PRT
<213> Artificial Sequence

<220>
<223> cMAb1 L chain aa

<400> 38

Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser
1 5 10 15

Gly Ala Tyr Gly Asp Ala Val Met Thr Gln Thr Pro Leu Ser Leu Pro
20 25 30

Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
35 40 45

Leu Glu Asn Ser Asn Gly Asn Thr Tyr Leu Asn Trp Tyr Phe Gln Lys
50 55 60

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe

65 70 75 80

Ser Gly Val Leu Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
85 90 95

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Phe Gly Val Tyr Phe
100 105 110

Cys Leu Gln Val Thr His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys
115 120 125

Leu Glu Ile Lys Arg Ala Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130 135 140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145 150 155 160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165 170 175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180 185 190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
195 200 205

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
210 215 220

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 39
<211> 1401
<212> DNA
<213> Artificial Sequence

<220>
<223> cMAb1-1 H chain na

<400> 39
atgaaacacc tgtggttctt cctcctgctg gtggcagctc ccagatgggt gctgagcgaa 60

gtgcagctgg tggagtctgg gggaggctta gtgaagcctg gagggtccct gaaactctcc 120

tgtgcagcct ctggattcac tttcagtacc tatgccatgt cttgggttcg ccagactccg 180

gagaagaggc tggagtgggt cgcaaccatt agtaatggtg gtacttacac ctactatcca 240

gacagtgtga agggtcgatt caccatctcc agagacaatg ccgagaacac cctgtacctg 300

caaatgagca gtctgaggtc tgaggacacg gccatgtatt tctgtgcaag actaatctac 360

tatgattacc ttgactactg gggccacggc accactctca cagtcagctc agcctccacc 420

aagggcccaa gcgtcttccc cctggcaccc tcctccaaga gcacctctgg cggcacagcc 480

gccctgggct gcctggtcaa ggactacttc cccgaacccg tgaccgtgag ctggaactca 540

ggcgccctga ccagcggcgt gcacaccttc cccgctgtcc tgcagtcctc aggactctac 600

tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc 660

aacgtgaatc acaagcccag caacaccaag gtggacaaga gagttgagcc caaatcttgt 720

gacaaaactc acacatgccc accctgccca gcacctgaac tcctgggggg accctcagtc 780

ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 840

tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 900

ggcgtggagg tgcataatgc caagacaaag ccccgggagg agcagtacaa cagcacgtac 960

cgggtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 1020

tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa 1080

ggccagcccc gggaaccaca ggtgtacacc ctgcccccat cccgggagga gatgaccaag 1140

aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag 1200

tgggagagca atggccagcc cgagaacaac tacaagacca cccctcccgt gctggactcc 1260

gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggc 1320

aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac ccagaagagc 1380

ctctccctgt ctcccggcaa a 1401

<210> 40
<211> 467
<212> PRT
<213> Artificial Sequence

<220>
<223> cMAb1-1 H chain aa

<400> 40

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1 5 10 15

Val Leu Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Thr Tyr Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu
50 55 60

Glu Trp Val Ala Thr Ile Ser Asn Gly Gly Thr Tyr Thr Tyr Tyr Pro
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Glu Asn
85 90 95

```
Thr Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met
            100                 105                 110

Tyr Phe Cys Ala Arg Leu Ile Tyr Tyr Asp Tyr Leu Asp Tyr Trp Gly
        115                 120                 125

His Gly Thr Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
    130                 135                 140

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
145                 150                 155                 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
                165                 170                 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
            180                 185                 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
        195                 200                 205

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
    210                 215                 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys
225                 230                 235                 240

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365
```

```
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Lys
465
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<210> 41
<211> 1389
<212> DNA
<213> Artificial Sequence

<220>
<223> cMAb1-2 H chain na

<400> 41

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tgtgcagcct ctggattcac tttcagtacc tatgccatgt cttgggttcg ccagactccg      180

gagaagaggc tggagtgggt cgcaaccatt agtaatggtg gtacttacac ctactatcca      240

gacagtgtga agggtcgatt caccatctcc agagacaatg ccgagaacac cctgtacctg      300

caaatgagca gtctgaggtc tgaggacacg gccatgtatt tctgtgcaag actaatctac      360

tatgattacc ttgactactg gggccacggc accactctca cagtcagctc agccagcacc      420

aagggccctt ccgtgttccc tctggccccc tgtagccgtt ccaccagcga gtccaccgcc      480

gcccttggct gtctggtgaa ggactacttc cctgagcctg tgaccgtgag ctggaactcc      540

ggagccctta ccagcggcgt gcacaccttc cctgccgtgc tgcagtccag cggcctttac      600

tccctgagct ccgtggtgac cgtgcctagc tccaacttcg gcacccaaac ctacacctgt      660

aacgtggacc acaagcctag caacaccaag gtggacaaga ccgtggagcg taagtgttgt      720

gtggagtgtc ctccttgtcc tgcccctcct gtggccggac cttccgtgtt ccttttccct      780

cctaagccta aggacaccct gatgatcagc cgtacccctg aggtgacctg tgtggtggtg      840
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gacgtgtccc acgaggaccc tgaggtgcag ttcaactggt acgtggacgg cgtggaggtg 900

cacaacgcca agaccaagcc tcgtgaggag caattcaaca gcaccttccg tgtggtgtcc 960

gtgcttaccg tggtgcacca agactggctg aacggcaagg agtacaagtg taaggtgagc 1020

aacaagggac ttcctgcccc tatcgagaag accatctcca agaccaaggg ccaacctcgt 1080

gagcctcaag tgtacaccct tcctcctagc cgtgaggaga tgaccaagaa ccaagtgtcc 1140

cttacctgtc tggtgaaggg cttctaccct agcgacatcg ccgtggagtg ggagtccaac 1200

ggacaacctg agaacaacta caagaccacc cctcctatgc ttgacagcga cggctccttc 1260

ttcctgtaca gcaagctgac cgtggacaag tcccgttggc aacaaggcaa cgtgttcagc 1320

tgttccgtga tgcacgaggc cctgcacaac cactacaccc aaaagagcct ttccctgagc 1380

cctggaaag 1389

<210> 42
<211> 463
<212> PRT
<213> Artificial Sequence

<220>
<223> cMAb1-2 H chain aa

<400> 42

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1 5 10 15

Val Leu Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Thr Tyr Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu
50 55 60

Glu Trp Val Ala Thr Ile Ser Asn Gly Gly Thr Tyr Thr Tyr Tyr Pro
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Glu Asn
85 90 95

Thr Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met
100 105 110

Tyr Phe Cys Ala Arg Leu Ile Tyr Tyr Asp Tyr Leu Asp Tyr Trp Gly
115 120 125

His Gly Thr Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
130 135 140

```
Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
145                 150                 155                 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
                165                 170                 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
            180                 185                 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
        195                 200                 205

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
    210                 215                 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys
225                 230                 235                 240

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
                245                 250                 255

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            260                 265                 270

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        275                 280                 285

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    290                 295                 300

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
305                 310                 315                 320

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                325                 330                 335

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
            340                 345                 350

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        355                 360                 365

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    370                 375                 380

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385                 390                 395                 400

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser
                405                 410                 415
```

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
420 425 430

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
435 440 445

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 43
<211> 15
<212> DNA
<213> Mus musculus

<400> 43
acctatgcca tgtct 15

<210> 44
<211> 5
<212> PRT
<213> Mus musculus

<400> 44

Thr Tyr Ala Met Ser
1 5

<210> 45
<211> 51
<212> DNA
<213> Mus musculus

<400> 45
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<210> 46
<211> 17
<212> PRT
<213> Mus musculus

<400> 46

Thr Ile Ser Asn Gly Gly Thr Tyr Thr Tyr Tyr Pro Asp Ser Val Lys
1 5 10 15

Gly

<210> 47
<211> 27
<212> DNA
<213> Mus musculus

<400> 47
ctaatctact atgattacct tgactac 27

<210> 48

<211> 9
<212> PRT
<213> Mus musculus

<400> 48

Leu Ile Tyr Tyr Asp Tyr Leu Asp Tyr
1 5

<210> 49
<211> 48
<212> DNA
<213> Mus musculus

<400> 49
aggtctagtc agagccttga aaacagtaat ggaaacacct atttgaac 48

<210> 50
<211> 16
<212> PRT
<213> Mus musculus

<400> 50

Arg Ser Ser Gln Ser Leu Glu Asn Ser Asn Gly Asn Thr Tyr Leu Asn
1 5 10 15

<210> 51
<211> 21
<212> DNA
<213> Mus musculus

<400> 51
agggtttcca accgattttc t 21

<210> 52
<211> 7
<212> PRT
<213> Mus musculus

<400> 52

Arg Val Ser Asn Arg Phe Ser
1 5

<210> 53
<211> 27
<212> DNA
<213> Mus musculus

<400> 53
ctccaagtta ctcatgtccc gtggacg 27

<210> 54
<211> 9
<212> PRT
<213> Mus musculus

<400> 54

Leu Gln Val Thr His Val Pro Trp Thr

1 5

<210> 55
<211> 1389
<212> DNA
<213> Artificial Sequence

<220>
<223> hMAb1-T1H

<400> 55
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gtgcagctgg tggaaagcgg cggaggcctg gtgcagcctg gcggctctct gagactgagc 120

tgtgccgcca gcggcttcac cttcagcacc tacgccatga gctgggtccg acaggcccct 180

ggcaagggac tggaatgggt ggcaaccatc agcaacggcg gcacctacac ctactacccc 240

gacagcgtga agggccggtt caccatcagc cgggacaacg ccaagaacac cctgtacctg 300

cagatgaaca gcctgcgggc cgaggacacc gccgtgtact actgcgccag actgatctac 360

tacgactacc tggactactg gggccagggc accctggtca ccgtcagctc agccagcacc 420

aagggccctt ccgtgttccc tctggccccc tgtagccgtt ccaccagcga gtccaccgcc 480

gcccttggct gtctggtgaa ggactacttc cctgagcctg tgaccgtgag ctggaactcc 540

ggagccctta ccagcggcgt gcacaccttc cctgccgtgc tgcagtccag cggcctttac 600

tccctgagct ccgtggtgac cgtgcctagc tccaacttcg gcacccaaac ctacacctgt 660

aacgtggacc acaagcctag caacaccaag gtggacaaga ccgtggagcg taagtgttgt 720

gtggagtgtc ctccttgtcc tgcccctcct gtggccggac cttccgtgtt ccttttccct 780

cctaagccta aggacaccct gatgatcagc cgtacccctg aggtgacctg tgtggtggtg 840

gacgtgtccc acgaggaccc tgaggtgcag ttcaactggt acgtggacgg cgtggaggtg 900

cacaacgcca agaccaagcc tcgtgaggag caattcaaca gcaccttccg tgtggtgtcc 960

gtgcttaccg tggtgcacca agactggctg aacggcaagg agtacaagtg taaggtgagc 1020

aacaagggac ttcctgcccc tatcgagaag accatctcca agaccaaggg ccaacctcgt 1080

gagcctcaag tgtacaccct tcctcctagc cgtgaggaga tgaccaagaa ccaagtgtcc 1140

cttacctgtc tggtgaaggg cttctaccct agcgacatcg ccgtggagtg ggagtccaac 1200

ggacaacctg agaacaacta caagaccacc cctcctatgc ttgacagcga cggctccttc 1260

ttcctgtaca gcaagctgac cgtggacaag tcccgttggc aacaaggcaa cgtgttcagc 1320

tgttccgtga tgcacgaggc cctgcacaac cactacaccc aaaagagcct ttccctgagc 1380

cctggaaag 1389

<210> 56
<211> 463
<212> PRT
<213> Artificial Sequence

<220>

<223> hMAb1-T1H aa

<400> 56

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Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1               5                  10                  15

Val Leu Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
            20                  25                  30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
        35                  40                  45

Ser Thr Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
    50                  55                  60

Glu Trp Val Ala Thr Ile Ser Asn Gly Gly Thr Tyr Thr Tyr Tyr Pro
65                  70                  75                  80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
                85                  90                  95

Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Arg Leu Ile Tyr Tyr Asp Tyr Leu Asp Tyr Trp Gly
        115                 120                 125

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
    130                 135                 140

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
145                 150                 155                 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
                165                 170                 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
            180                 185                 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
        195                 200                 205

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
    210                 215                 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys
225                 230                 235                 240

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
                245                 250                 255
```

```
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            260                 265                 270

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        275                 280                 285

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    290                 295                 300

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
305                 310                 315                 320

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                325                 330                 335

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
            340                 345                 350

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        355                 360                 365

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    370                 375                 380

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385                 390                 395                 400

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser
                405                 410                 415

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
            420                 425                 430

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        435                 440                 445

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455                 460
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<210> 57
<211> 1389
<212> DNA
<213> Artificial Sequence

<220>
<223> hMAb1-T2H na

<400> 57
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tgtgccgcca gcggcttcac cttcagcacc tacgccatga gctgggtccg acaggccccct 180

ggcaagggac tggaatgggt ggcaaccatc agccaaggcg gcacctacac ctactacccc 240

gacagcgtga agggccggtt caccatcagc cgggacaacg ccaagaacac cctgtacctg 300

cagatgaaca gcctgcgggc cgaggacacc gccgtgtact actgcgccag actgatctac 360

tacgactacc tggactactg gggccagggc accctggtca ccgtcagctc agccagcacc 420

aagggccctt ccgtgttccc tctggccccc tgtagccgtt ccaccagcga gtccaccgcc 480

gcccttggct gtctggtgaa ggactacttc cctgagcctg tgaccgtgag ctggaactcc 540

ggagccctta ccagcggcgt gcacaccttc cctgccgtgc tgcagtccag cggcctttac 600

tccctgagct ccgtggtgac cgtgcctagc tccaacttcg gcacccaaac ctacacctgt 660

aacgtggacc acaagcctag caacaccaag gtggacaaga ccgtggagcg taagtgttgt 720

gtggagtgtc ctccttgtcc tgcccctcct gtggccggac cttccgtgtt ccttttccct 780

cctaagccta aggacaccct gatgatcagc cgtacccctg aggtgacctg tgtggtggtg 840

gacgtgtccc acgaggaccc tgaggtgcag ttcaactggt acgtggacgg cgtggaggtg 900

cacaacgcca agaccaagcc tcgtgaggag caattcaaca gcaccttccg tgtggtgtcc 960

gtgcttaccg tggtgcacca agactggctg aacggcaagg agtacaagtg taaggtgagc 1020

aacaagggac ttcctgcccc tatcgagaag accatctcca agaccaaggg ccaacctcgt 1080

gagcctcaag tgtacaccct tcctcctagc cgtgaggaga tgaccaagaa ccaagtgtcc 1140

cttacctgtc tggtgaaggg cttctaccct agcgacatcg ccgtggagtg ggagtccaac 1200

ggacaacctg agaacaacta caagaccacc cctcctatgc ttgacagcga cggctccttc 1260

ttcctgtaca gcaagctgac cgtggacaag tcccgttggc aacaaggcaa cgtgttcagc 1320

tgttccgtga tgcacgaggc cctgcacaac cactacaccc aaaagagcct ttccctgagc 1380

cctggaaag 1389

<210> 58
<211> 463
<212> PRT
<213> Artificial Sequence

<220>
<223> hMAb1-T2H aa

<400> 58

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1 5 10 15

Val Leu Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Thr Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu

```
    50                  55                  60

Glu Trp Val Ala Thr Ile Ser Gln Gly Gly Thr Tyr Thr Tyr Tyr Pro
65                  70                  75                  80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
                85                  90                  95

Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Arg Leu Ile Tyr Tyr Asp Tyr Leu Asp Tyr Trp Gly
        115                 120                 125

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
    130                 135                 140

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
145                 150                 155                 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
                165                 170                 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
            180                 185                 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
        195                 200                 205

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
    210                 215                 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys
225                 230                 235                 240

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
                245                 250                 255

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            260                 265                 270

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        275                 280                 285

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    290                 295                 300

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
305                 310                 315                 320
```

```
Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                325                 330                 335

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
            340                 345                 350

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        355                 360                 365

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    370                 375                 380

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385                 390                 395                 400

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser
                405                 410                 415

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
            420                 425                 430

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        435                 440                 445

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455                 460
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<210> 59
<211> 1389
<212> DNA
<213> Artificial Sequence

<220>
<223> hMAb1-T3H na

<400> 59

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gtgcagctgg tggaaagcgg cggaggcctg gtgcagcctg gcggctctct gagactgagc      120

tgtgccgcca gcggcttcac cttcagcacc tacgccatga gctgggtccg acaggcccct      180

ggcaagcggc tggaatgggt ggcaaccatc agcaacggcg gcacctacac ctactacccc      240

gacagcgtga agggccggtt caccatcagc cgggacaacg ccaagaacac cctgtacctg      300

cagatgaaca gcctgcgggc cgaggacacc gccgtgtact actgcgccag actgatctac      360

tacgactacc tggactactg gggccagggc accctggtca ccgtcagctc agccagcacc      420

aagggccctt ccgtgttccc tctggccccc tgtagccgtt ccaccagcga gtccaccgcc      480

gcccttggct gtctggtgaa ggactacttc cctgagcctg tgaccgtgag ctggaactcc      540

ggagccctta ccagcggcgt gcacaccttc cctgccgtgc tgcagtccag cggcctttac      600

tccctgagct ccgtggtgac cgtgcctagc tccaacttcg gcacccaaac ctacacctgt      660
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aacgtggacc acaagcctag caacaccaag gtggacaaga ccgtggagcg taagtgttgt 720

gtggagtgtc ctccttgtcc tgcccctcct gtggccggac cttccgtgtt ccttttccct 780

cctaagccta aggacaccct gatgatcagc cgtacccctg aggtgacctg tgtggtggtg 840

gacgtgtccc acgaggaccc tgaggtgcag ttcaactggt acgtggacgg cgtggaggtg 900

cacaacgcca agaccaagcc tcgtgaggag caattcaaca gcaccttccg tgtggtgtcc 960

gtgcttaccg tggtgcacca agactggctg aacggcaagg agtacaagtg taaggtgagc 1020

aacaagggac ttcctgcccc tatcgagaag accatctcca agaccaaggg ccaacctcgt 1080

gagcctcaag tgtacaccct tcctcctagc cgtgaggaga tgaccaagaa ccaagtgtcc 1140

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ggacaacctg agaacaacta caagaccacc cctcctatgc ttgacagcga cggctccttc 1260

ttcctgtaca gcaagctgac cgtggacaag tcccgttggc aacaaggcaa cgtgttcagc 1320

tgttccgtga tgcacgaggc cctgcacaac cactacaccc aaaagagcct ttccctgagc 1380

cctggaaag 1389

<210> 60
<211> 463
<212> PRT
<213> Artificial Sequence

<220>
<223> hMAb1-T3H aa

<400> 60

Met Lys His Leu Trp Phe Phe Leu Leu Val Ala Ala Pro Arg Trp
1 5 10 15

Val Leu Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Thr Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Arg Leu
50 55 60

Glu Trp Val Ala Thr Ile Ser Asn Gly Gly Thr Tyr Thr Tyr Tyr Pro
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Leu Ile Tyr Tyr Asp Tyr Leu Asp Tyr Trp Gly
115 120 125

```
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
    130                 135                 140

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
145                 150                 155                 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
                165                 170                 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
            180                 185                 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
        195                 200                 205

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
    210                 215                 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys
225                 230                 235                 240

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
                245                 250                 255

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            260                 265                 270

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        275                 280                 285

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    290                 295                 300

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
305                 310                 315                 320

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                325                 330                 335

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
            340                 345                 350

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        355                 360                 365

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    370                 375                 380

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
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385 390 395 400

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser
405 410 415

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
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Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Thr Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Arg Leu
50 55 60

Glu Trp Val Ala Thr Ile Ser Gln Gly Gly Thr Tyr Thr Tyr Tyr Pro
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Leu Ile Tyr Tyr Asp Tyr Leu Asp Tyr Trp Gly
115 120 125

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
130 135 140

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
145 150 155 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
165 170 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
180 185 190

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Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
        195                 200                 205

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
    210                 215                 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys
225                 230                 235                 240

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
                245                 250                 255

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            260                 265                 270

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        275                 280                 285

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    290                 295                 300

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
305                 310                 315                 320

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                325                 330                 335

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
            340                 345                 350

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        355                 360                 365

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    370                 375                 380

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385                 390                 395                 400

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser
                405                 410                 415

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
            420                 425                 430

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
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atcagctgca gaagcagcca gagcctggaa aacagcaacg gcaacaccta cctgaactgg 180

tatctgcaga agcccggcca gtcccccag ctgctgatct accgggtgtc caaccggttc 240

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tggaccttcg gccctggcac caaggtggac atcaagcgta cggtggccgc cccctccgtg 420

ttcatcttcc cccctccga cgagcagctg aagtccggca ccgcctccgt ggtgtgcctg 480

ctgaataact tctaccccag agaggccaag gtgcagtgga aggtggacaa cgccctgcag 540

tccgggaact cccaggagag cgtgaccgag caggacagca aggacagcac ctacagcctg 600

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Val Thr Leu Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
35 40 45

Leu Glu Asn Ser Asn Gly Asn Thr Tyr Leu Asn Trp Tyr Leu Gln Lys
50 55 60

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe
65 70 75 80

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe

85 90 95

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
100 105 110

Cys Leu Gln Val Thr His Val Pro Trp Thr Phe Gly Pro Gly Thr Lys
115 120 125

Val Asp Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130 135 140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145 150 155 160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165 170 175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180 185 190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
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Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
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tatctgcaga agcccggcca gtcccccag ctgctgatct accgggtgtc caaccggttc 240

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Val Thr Leu Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
35 40 45

Leu Glu Asn Glu Asn Lys Asn Leu Tyr Leu Asn Trp Tyr Leu Gln Lys
50 55 60

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe
65 70 75 80

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
85 90 95

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
100 105 110

Cys Leu Gln Val Thr His Val Pro Trp Thr Phe Gly Pro Gly Thr Lys
115 120 125

Val Asp Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130 135 140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145 150 155 160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165 170 175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180 185 190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
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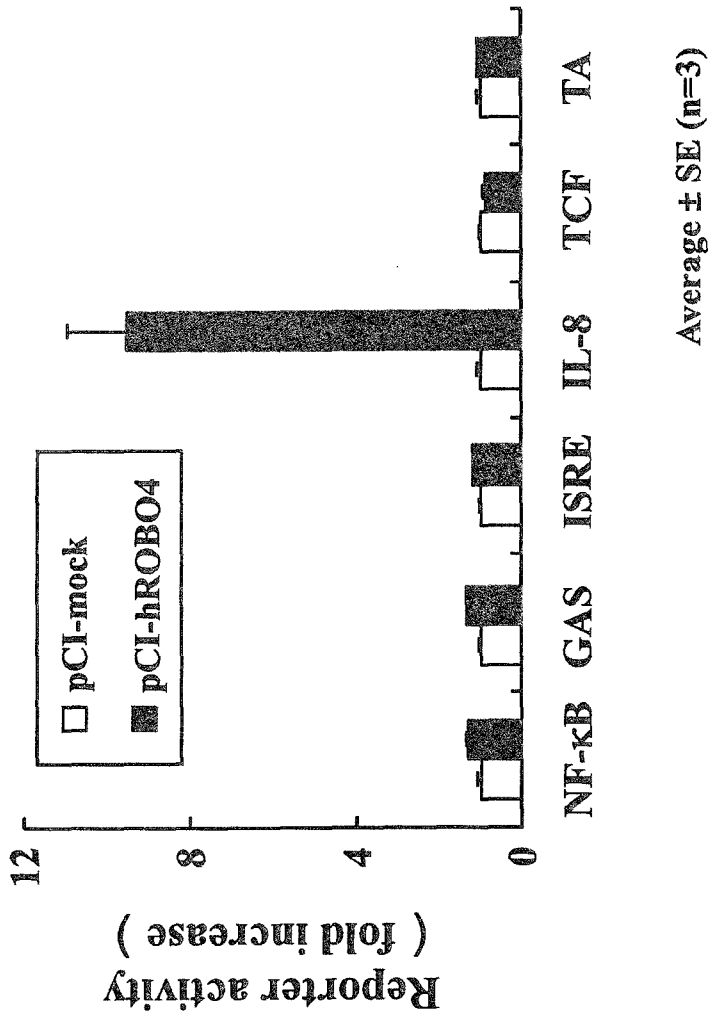
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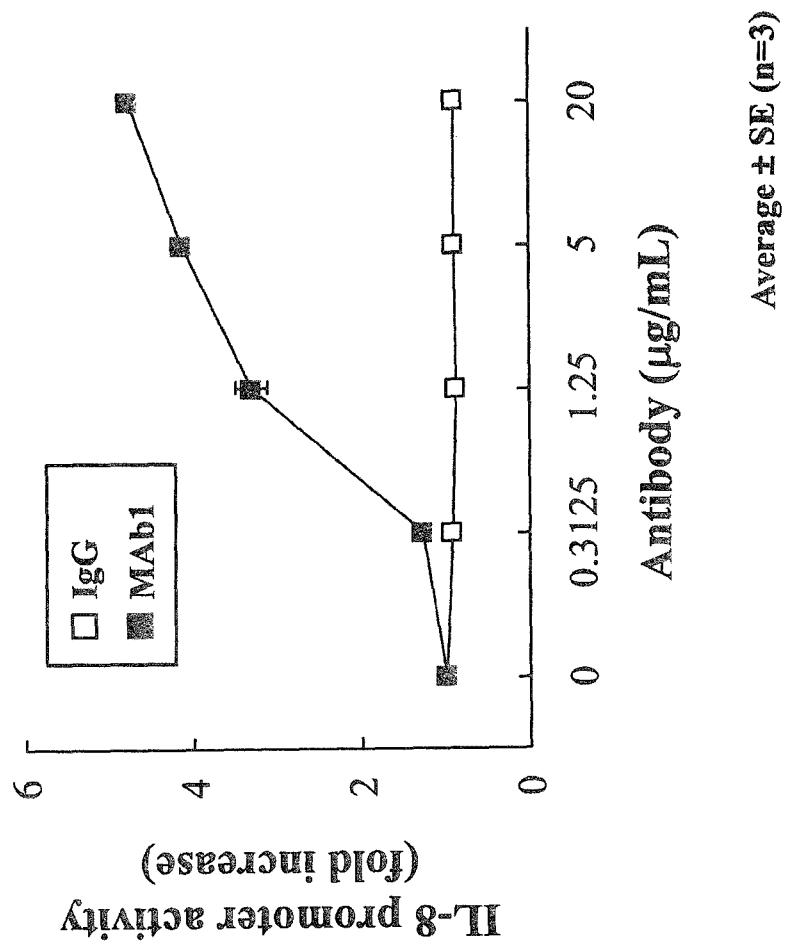
[Figure 1] Reporter activity



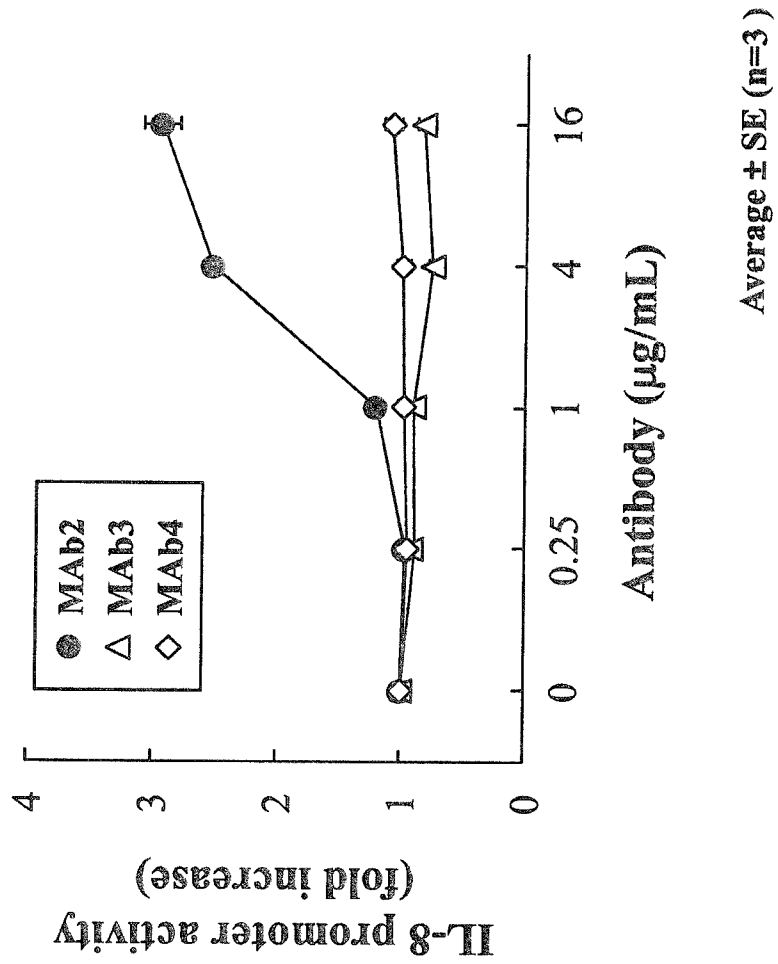
[Figure 2] IL-8 promoter activity



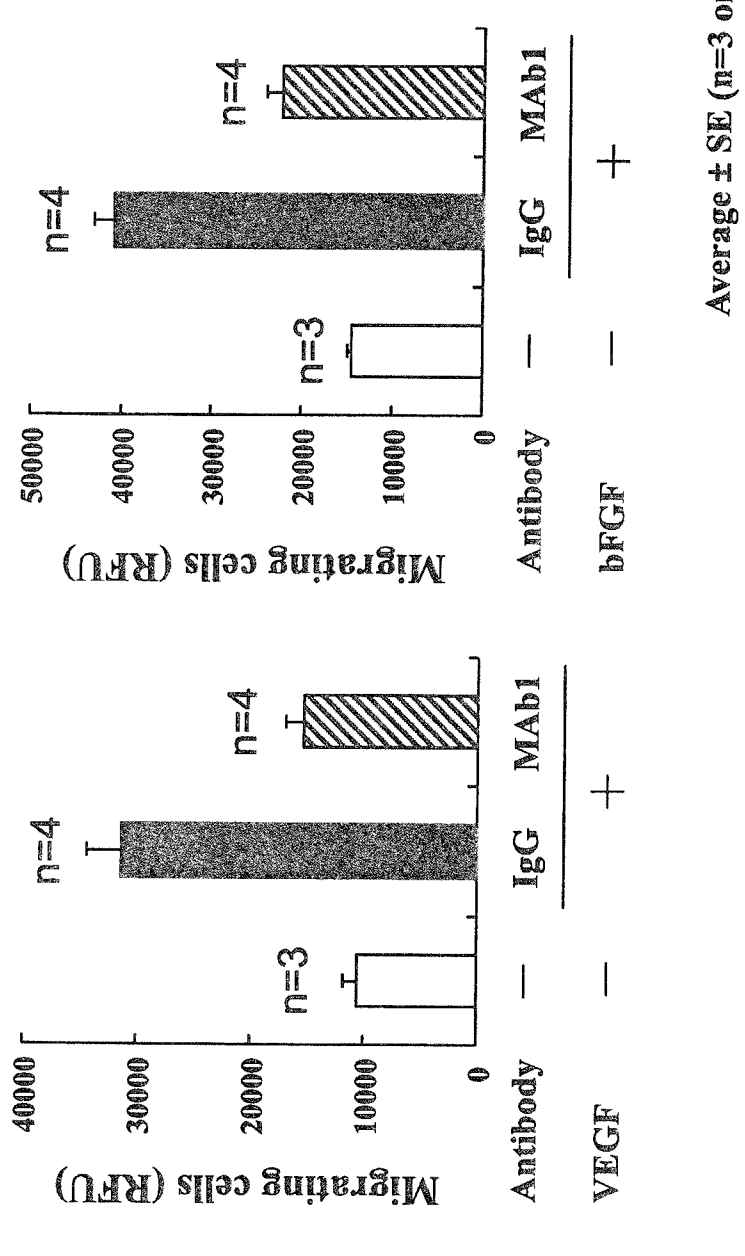
[Figure 3] IL-8 promoter activity



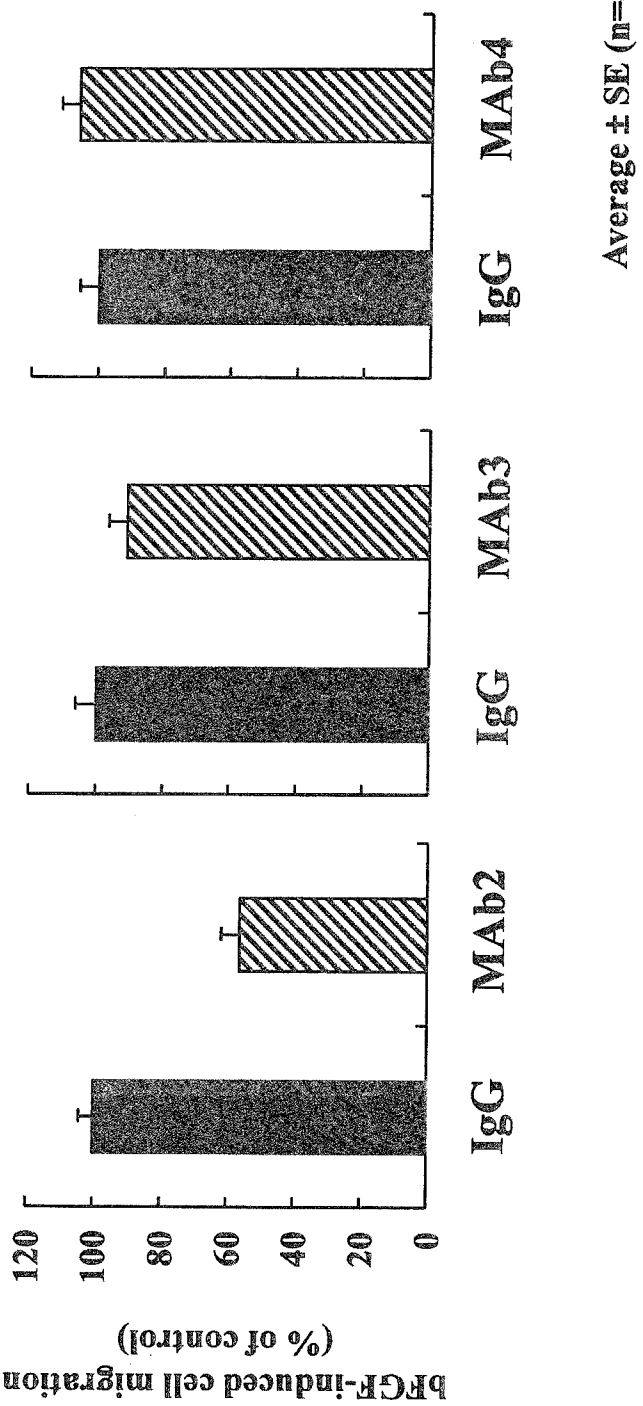
[Figure 4] IL-8 promoter activity



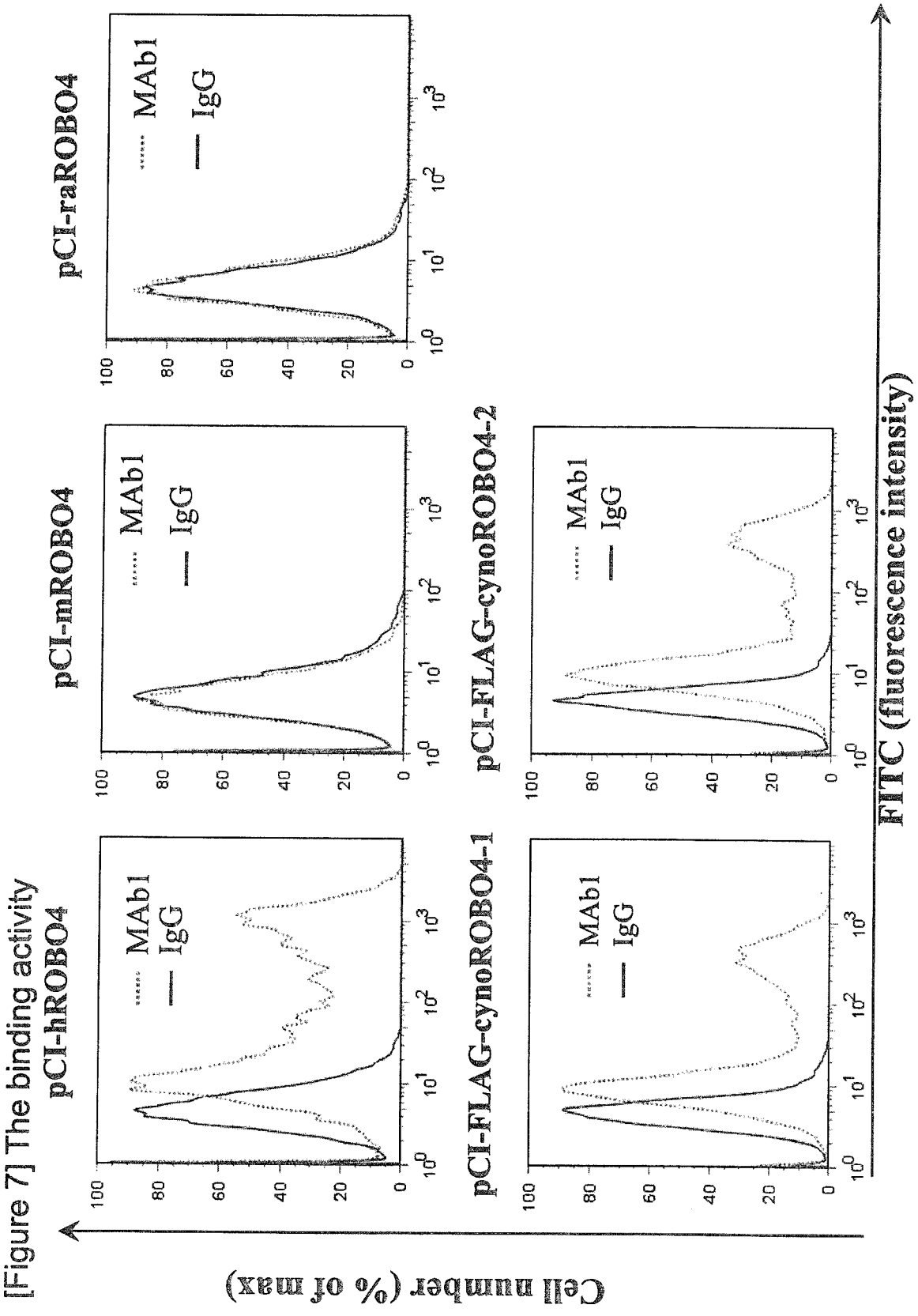
[Figure 5] The migratory capacity of HUVEC



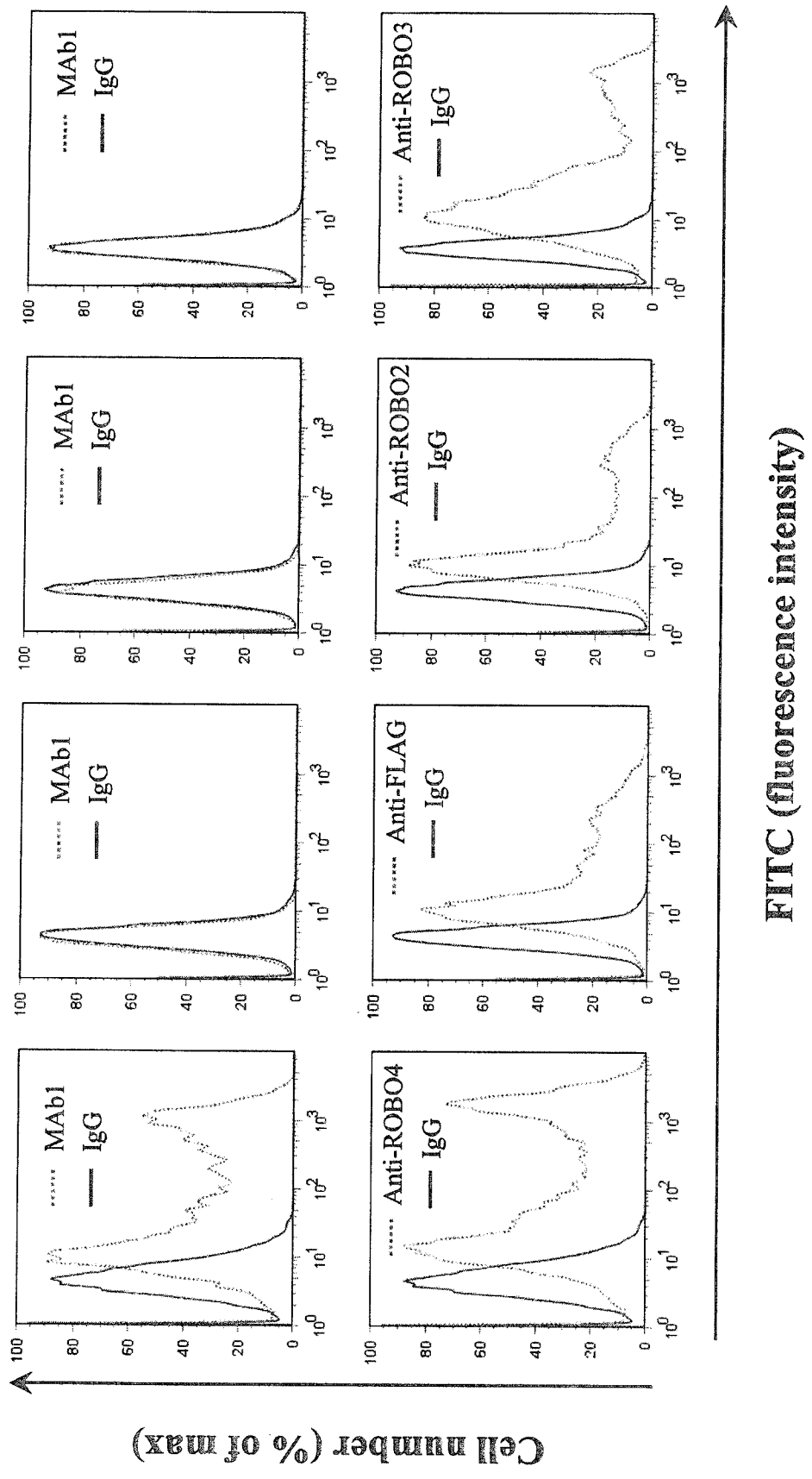
[Figure 6] The migratory capacity of HUVEC



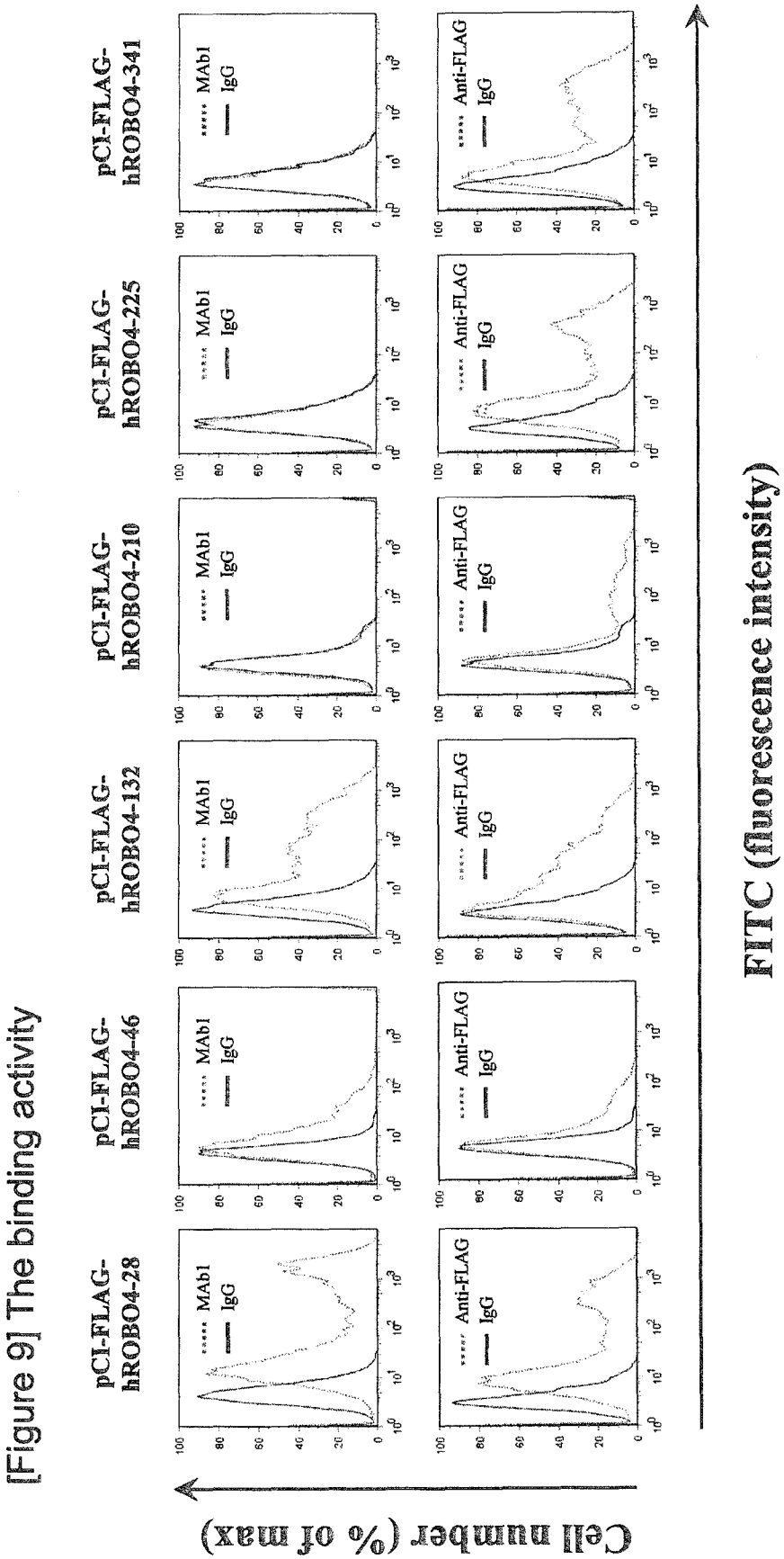
[Figure 7] The binding activity



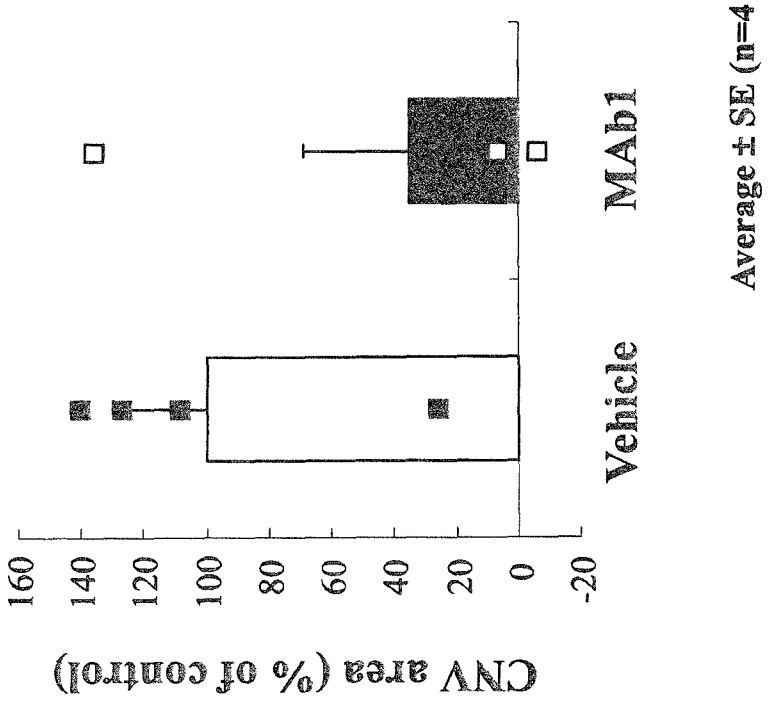
[Figure 8] The binding activity



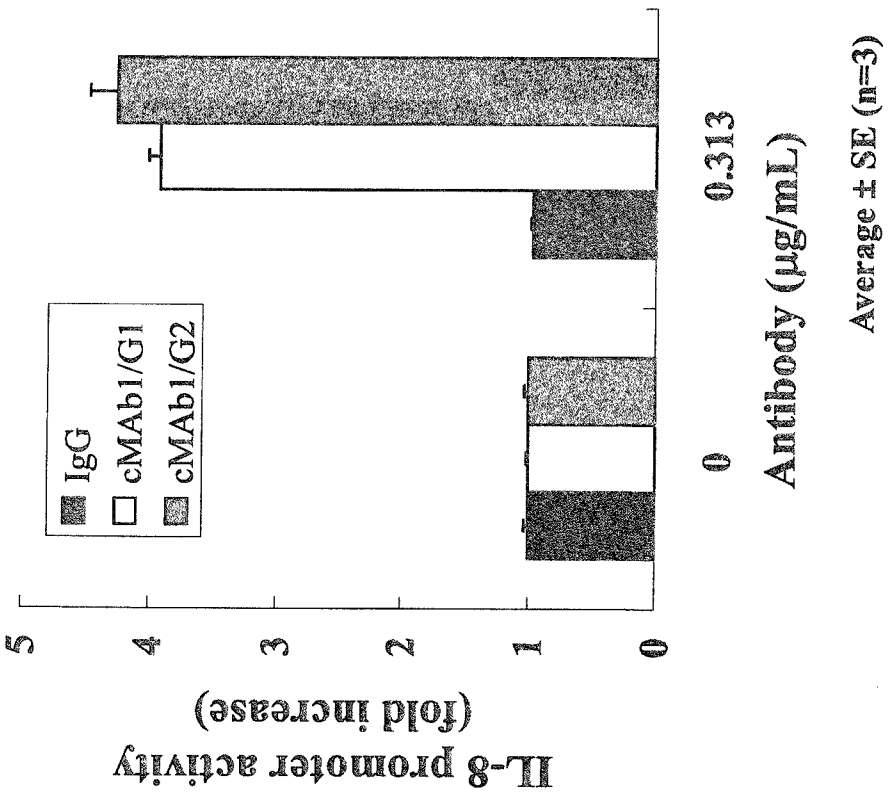
[Figure 9] The binding activity



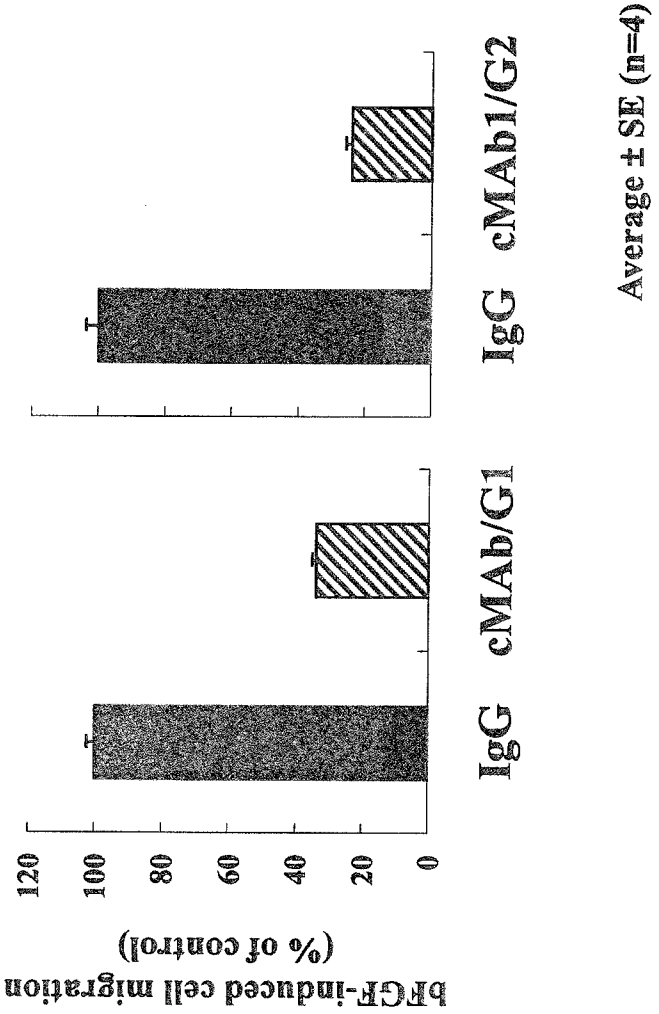
[Figure 10] In vivo efficacy of MAb1



[Figure 11] IL-8 promoter activity



[Figure 12] The migratory capacity of HUVEC



【Figure 13】 cDNA encoding full-length human ROBO4 (SEQ ID NO: 1)

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stop codon

[Figure 14] The full-length amino acid sequence of human ROBO4 (SEQ ID NO: 2)
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SHTQRLGRGMPPWPPDSQISSQRSQLHCRMPKAGASPVDYS

[Figure 15] The nucleotide sequence of cDNA encoding the heavy chain variable region of MAb1 (SEQ ID NO: 30)
gaagtgcagctggtggagtctgggggaggcttagtgaagcctggagggtccctgaaactctcctgtgcagcctctggattcactttca
gtacctatgccatgtcttgggttcgccagactccggagaagaggctggagtgggtcgcaaccattagtaatggtggtacttacacct
actatccagacagtgtgaagggtcgattcaccatctccagagacaatgccgagaacaccctgtacctgcaaatgagcagtctgagg
tctgaggacacggccatgtatttctgtgcaagactaatctactatgattaccttgactactggggccacggcaccactctcacagtctc
ctca

[Figure 16] The amino acid sequence of the MAb1 heavy chain variable region (SEQ ID NO: 31)
EVQLVESGGGLVKPGGSLKLSCAASGFTFSTYAMSWVRQTPEKRLEWVATISNGGTYTY

YPDSVKGRFTISRDNAENTLYLQMSSLRSEDTAMYFCARLIYYDYLDYWGHGTTLTVSS

[Figure 17] The nucleotide sequence of cDNA encoding the light chain variable region of MAb1 (SEQ ID NO: 32)

gatgctgtgatgacccaaactccactctccctgcctgtcagtcttggagatcaagcctccatctcttgcaggtctagtcagagccttga
aaacagtaatggaaacacctatttgaactggtacttccagaaaccaggccagtctccacagctcctgatctacagggtttccaaccg
attttctggggtcctagacaggttcagtggtagtggatcagggacagatttcacactgaaaatcagcagagtggaggctgaagatt
tcggagtttatttctgcctccaagttactcatgtcccgtggacgttcggtggaggcaccaagctggaaatcaaacgggct

[Figure 18] The amino acid sequence of the MAb1 light chain variable region (SEQ ID NO: 33)

DAVMTQTPLSLPVSLGDQASISCRSSQSLENSNGNTYLNWYFQKPGQSPQLLIYRVS
NRFSGVLDRFSGSGSGTDFTLKISRVEAEDFGVYFCLQVTHVPWTFGGGTKLEIKRA

[Figure 19] Figure 19 shows the nucleotide sequence of cDNA encoding the light chain of cMAb1 (SEQ ID NO: 37)

atggtgctgcagacccaggtgttcatctccctgctgctgtggatctccggcgcgtacggcgatgctgtgatgacccaaactccactctc
cctgcctgtcagtcttggagatcaagcctccatctcttgcaggtctagtcagagccttgaaaacagtaatggaaacacctatttgaac
tggtacttccagaaaccaggccagtctccacagctcctgatctacagggtttccaaccgattttctggggtcctagacaggttcagtgg
tagtggatcagggacagatttcacactgaaaatcagcagagtggaggctgaagatttcggagtttatttctgcctccaagttactca
tgtcccgtggacgttcggtggaggcaccaagctggaaatcaaacgggctgtggccgcccctcgtgttcatcttcccccctccgacg
agcagctgaagtccggcaccgcctccgtggtgtgcctgctgaataacttctaccccagagaggccaaggtgcagtggaaggtggac
aacgccctgcagtccgggaactcccaggagagcgtgaccgagcaggacagcaaggacagcacctacagcctgagcagcaccctga
ccctgagcaaagccgactacgagaagcacaaggtgtacgcctgcgaggtgacccaccagggcctgagctcccccgtcaccaagagc
ttcaacaggggggagtgt

Secretory signal sequence(1-60), Variable region(61-402), Constant region(403-717)

[Figure 20] The amino acid sequence of the cMAb1 light chain (SEQ ID NO: 38)

MVLQTQVFISLLLWISGAYGDAVMTQTPLSLPVSLGDQASISCRSSQSLENSNGNTYLNW
YFQKPGQSPQLLIYRVSNRFSGVLDRFSGSGSGTDFTLKISRVEAEDFGVYFCLQVTHVP
WTFGGGTKLEIKRAVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C

Secretory signal sequence(1-20), Variable region(21-134), Constant region(135-239)

[Figure 21] The nucleotide sequence of cDNA encoding the heavy chain of cMAb1-1 (SEQ ID NO: 39)

atgaaacacctgtggttcttcctcctgctggtggcagctcccagatgggtgctgagcgaagtgcagctggtggagtctgggggg
aggcttagtgaagcctggagggtccctgaaactctcctgtgcagcctctggattcactttcagtacctatgccatgtcttgggttc
gccagactccggagaagaggctggagtgggtcgcaaccattagtaatggtggtacttacacctactatccagacagtgtgaa
gggtcgattcaccatctccagagacaatgccgagaacaccctgtacctgcaaatgagcagtctgaggtctgaggacacggcc
atgtatttctgtgcaagactaatctactatgattaccttgactactggggccacggcaccactctcacagtcagctcagcctcca
ccaagggcccaagcgtcttccccctggcaccctcctccaagagcacctctggcggcacagccgccctgggctgcctggtcaagg
actacttccccgaacccgtgaccgtgagctggaactcaggcgccctgaccagcggcgtgcacaccttccccgctgtcctgcagtc
ctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctacatctgcaacgtgaatca
caagcccagcaacaccaaggtggacaagagagttgagcccaaatcttgtgacaaaactcacacatgcccaccctgcccagca
cctgaactcctgggggggaccctcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtca
catgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgc
caagacaaagccccgggaggagcagtacaacagcacgtaccgggtggtcagcgtcctcaccgtcctgcaccaggactggctg
aatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaaggc
cagccccgggaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctgg
tcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatggccagcccgagaacaactacaagaccacccctcc
cgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcagggcaacgtcttctc
atgctccgtgatgcatgaggctctgcacaaccactacacccagaagagcctctccctgtctcccggcaaa

Secretory signal sequence(1-57), Variable region(58-411), Constant region(412-1401)

[Figure 22] The amino acid sequence of the cMAb1-1 heavy chain (SEQ ID NO: 40)

MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVKPGGSLKLSCAASGFTFSTYAMSWV
RQTPEKRLEWVATISNGGTYTYYPDSVKGRFTISRDNAENTLYLQMSSLRSEDTAMY
FCARLIYYDYLDYWGHGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN
TKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
HNHYTQKSLSLSPGK

Secretory signal sequence(1-19), Variable region(20-137), Constant region(138-467)

[Figure 23] The nucleotide sequence of cDNA encoding the heavy chain of cMAb1-2 (SEQ ID NO: 41)

atgaaacacctgtggttcttcctcctgctggtggcagctcccagatgggtgctgagcgaagtgcagctggtggagtctgggggaggc
ttagtgaagcctggagggtccctgaaactctcctgtgcagcctctggattcactttcagtacctatgccatgtcttgggttcgccagact
ccggagaagaggctggagtgggtcgcaaccattagtaatggtggtacttacacctactatccagacagtgtgaagggtcgattcac
catctccagagacaatgccgagaacaccctgtacctgcaaatgagcagtctgaggtctgaggacacggccatgtatttctgtgcaag
actaatctactatgattaccttgactactggggccacggcaccactctcacagtcagctcagccagcaccaagggcccttccgtgttcc
ctctggcccttgtagccgttccaccagcgagtccaccgccgcccttggctgtctggtgaaggactacttccctgagcctgtgaccgtga
gctggaactccggagcccttaccagcggcgtgcacaccttccctgccgtgctgcagtccagcggcctttactccctgagctccgtggtg
accgtgcctagctccaacttcggcacccaaacctacacctgtaacgtggaccacaagcctagcaacaccaaggtggacaagaccgt
ggagcgtaagtgttgtgtggagtgtcctccttgtcctgcccctcctgtggccggaccttccgtgttccttttccctcctaagcctaaggac
accctgatgatcagccgtacccctgaggtgacctgtgtggtggtggacgtgtcccacgaggaccctgaggtgcagttcaactggtac
gtggacggcgtggaggtgcacaacgccaagaccaagcctcgtgaggagcaattcaacagcaccttccgtgtggtgtccgtgcttacc
gtggtgcaccaagactggctgaacggcaaggagtacaagtgtaaggtgagcaacaagggacttcctgcccctatcgagaagacca
tctccaagaccaagggccaacctcgtgagcctcaagtgtacacccttcctcctagccgtgaggagatgaccaagaaccaagtgtccc
ttacctgtctggtgaagggcttctaccctagcgacatcgccgtggagtgggagtccaacggacaacctgagaacaactacaagacc
acccctcctatgcttgacagcgacggctccttcttcctgtacagcaagctgaccgtggacaagtcccgttggcaacaaggcaacgtgtt
cagctgttccgtgatgcacgaggccctgcacaaccactacacccaaaagagcctttccctgagccctggaaag

Secretory signal sequence(1-57), Variable region(58-411), Constant region(412-1389)

[Figure 24] The amino acid sequence of the cMAb1-2 heavy chain (SEQ ID NO: 42)

MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVKPGGSLKLSCAASGFTFSTYAMSWV
RQTPEKRLEWVATISNGGTYTYYPDSVKGRFTISRDNAENTLYLQMSSLRSEDTAMY
FCARLIYYDYLDYWGHGTTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSN
TKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKV
SNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK

Secretory signal sequence(1-19), Variable region(20-137), Constant region(138-463)

[Figure 25] The amino acid sequence of the heavy chain CDRH1 of MAb1 (SEQ ID NO: 44)
TYAMS

[Figure 26]The amino acid sequence of the heavy chain CDRH2 of MAb1 (SEQ ID NO: 46)
TISNGGTYTYYPDSVKG

[Figure 27] The amino acid sequence of the heavy chain CDRH3 of MAb1 (SEQ ID NO: 48)
LIYYDYLDY

[Figure 28] The amino acid sequence of the light chain CDRL1 of MAb1 (SEQ ID NO: 50)
RSSQSLENSNGNTYLN

[Figure 29] The amino acid sequence of the light chain CDRL2 of MAb1 (SEQ ID NO: 52)
RVSNRFS

[Figure 30] The amino acid sequence of the light chain CDRL3 of MAb1 (SEQ ID NO: 54)
LQVTHVPWT

[Figure 31] The nucleotide sequence of cDNA encoding a hMAb1-H1-type heavy chain (SEQ ID NO: 55).

atgaaacacctgtggttcttcctcctgctggtggcagctcccagatgggtgctgagcgaggtgcagctggtggaaagcg
gcggaggcctggtgcagcctggcggctctctgagactgagctgtgccgccagcggcttcaccttcagcacctacgccatg
agctgggtccgacaggcccctggcaagggactggaatgggtggcaaccatcagcaacggcggcacctacacctactac
cccgacagcgtgaagggccggttcaccatcagccgggacaacgccaagaacaccctgtacctgcagatgaacagcctg
cgggccgaggacaccgccgtgtactactgcgccagactgatctactacgactacctggactactggggccagggcaccc
tggtcaccgtcagctcagccagcaccaagggcccttccgtgttccctctggcccccttgtagccgttccaccagcgagtccac
cgccgcccttggctgtctggtgaaggactacttccctgagcctgtgaccgtgagctggaactccggagcccttaccagcg
gcgtgcacaccttccctgccgtgctgcagtccagcggcctttactccctgagctccgtggtgaccgtgcctagctccaactt
cggcacccaaacctacacctgtaacgtggaccacaagcctagcaacaccaaggtggacaagaccgtggagcgtaagt
gttgtgtggagtgtcctccttgtcctgcccctcctgtggccggaccttccgtgttccttttccctcctaagcctaaggacaccc
tgatgatcagccgtacccctgaggtgacctgtgtggtggtggacgtgtcccacgaggaccctgaggtgcagttcaactg
gtacgtggacggcgtggaggtgcacaacgccaagaccaagcctcgtgaggagcaattcaacagcaccttccgtgtggt
gtccgtgcttaccgtggtgcaccaagactggctgaacggcaaggagtacaagtgtaaggtgagcaacaagggacttcc
tgcccctatcgagaagaccatctccaagaccaagggccaacctcgtgagcctcaagtgtacacccttcctcctagccgtg
aggagatgaccaagaaccaagtgtcccttacctgtctggtgaagggcttctaccctagcgacatcgccgtggagtggga
gtccaacggacaacctgagaacaactacaagaccacccctcctatgcttgacagcgacggctccttcttcctgtacagca
agctgaccgtggacaagtcccgttggcaacaaggcaacgtgttcagctgttccgtgatgcacgaggccctgcacaacca
ctacacccaaaagagcctttccctgagccctggaaag

Secretory signal sequence(1-57),Variable region (58-411),Constant region(412-1389)

[Figure 32] The amino acid sequence of the hMAb1-H1-type heavy chain (SEQ ID NO: 56)

MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMS
WVRQAPGKGLEWVATISNGGTYTYYPDSVKGRFTISRDNAKNTLYLQMNSLRA
EDTAVYYCARLIYYDYLDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQT
YTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTV
VHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Secretory signal sequence(1-19), Variable region(20-137), Constant region(138-463)

[Figure 33] The nucleotide sequence of cDNA encoding a hMAb1-H2-type heavy chain (SEQ ID NO: 57).

atgaaacacctgtggttcttcctcctgctggtggcagctcccagatgggtgctgagcgaggtgcagctggtggaaagcg
gcggaggcctggtgcagcctggcggctctctgagactgagctgtgccgccagcggcttcaccttcagcacctacgccatg
agctgggtccgacaggcccctggcaagggactggaatgggtggcaaccatcagccaaggcggcacctacacctactac
cccgacagcgtgaagggccggttcaccatcagccgggacaacgccaagaacaccctgtacctgcagatgaacagcctg
cgggccgaggacaccgccgtgtactactgcgccagactgatctactacgactacctggactactggggccagggcaccc
tggtcaccgtcagctcagccagcaccaagggcccttccgtgttccctctggccccttgtagccgttccaccagcgagtccac
cgccgcccttggctgtctggtgaaggactacttccctgagcctgtgaccgtgagctggaactccggagcccttaccagcg
gcgtgcacaccttccctgccgtgctgcagtccagcggcctttactccctgagctccgtggtgaccgtgcctagctccaactt
cggcacccaaacctacacctgtaacgtggaccacaagcctagcaacaccaaggtggacaagaccgtggagcgtaagt
gttgtgtggagtgtcctccttgtcctgcccctcctgtggccggaccttccgtgttccttttccctcctaagcctaaggacaccc
tgatgatcagccgtacccctgaggtgacctgtgtggtggtggacgtgtcccacgaggaccctgaggtgcagttcaactg
gtacgtggacggcgtggaggtgcacaacgccaagaccaagcctcgtgaggagcaattcaacagcaccttccgtgtggt
gtccgtgcttaccgtggtgcaccaagactggctgaacggcaaggagtacaagtgtaaggtgagcaacaagggacttcc
tgcccctatcgagaagaccatctccaagaccaagggccaacctcgtgagcctcaagtgtacacccttcctcctagccgtg
aggagatgaccaagaaccaagtgtcccttacctgtctggtgaagggcttctaccctagcgacatcgccgtggagtggga
gtccaacggacaacctgagaacaactacaagaccacccctcctatgcttgacagcgacggctccttcttcctgtacagca
agctgaccgtggacaagtcccgttggcaacaaggcaacgtgttcagctgttccgtgatgcacgaggccctgcacaacca
ctacacccaaaagagcctttccctgagccctggaaag

Secretory signal sequence(1-57), Variable region(58-411), Constant region(412-1389)

[Figure 34] The amino acid sequence of the hMAb1-H2-type heavy chain (SEQ ID NO: 58).

MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMS
WVRQAPGKGLEWVATISQGGTYTYYPDSVKGRFTISRDNAKNTLYLQMNSLRA
EDTAVYYCARLIYYDYLDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQT
YTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTV
VHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Secretory signal sequence(1-19), Variable region(20-137), Constant region(138-463)

[Figure 35] The nucleotide sequence of cDNA encoding a hMAb1-H3-type heavy chain (SEQ ID NO: 59)

atgaaacacctgtggttcttcctcctgctggtggcagctcccagatgggtgctgagcgaggtgcagctggtggaaagcg
gcggaggcctggtgcagcctggcggctctctgagactgagctgtgccgccagcggcttcaccttcagcacctacgccatg
agctgggtccgacaggcccctggcaagcggctggaatgggtggcaaccatcagcaacggcggcacctacacctactac
cccgacagcgtgaagggccggttcaccatcagccgggacaacgccaagaacaccctgtacctgcagatgaacagcctg
cgggccgaggacaccgccgtgtactactgcgccagactgatctactacgactacctggactactggggccagggcaccc
tggtcaccgtcagctcagccagcaccaagggcccttccgtgttccctctggccccttgtagccgttccaccagcgagtccac
cgccgcccttggctgtctggtgaaggactacttccctgagcctgtgaccgtgagctggaactccggagcccttaccagcg
gcgtgcacaccttccctgccgtgctgcagtccagcggcctttactccctgagctccgtggtgaccgtgcctagctccaactt
cggcacccaaacctacacctgtaacgtggaccacaagcctagcaacaccaaggtggacaagaccgtggagcgtaagt
gttgtgtggagtgtcctccttgtcctgcccctcctgtggccggaccttccgtgttccttttccctcctaagcctaaggacaccc
tgatgatcagccgtacccctgaggtgacctgtgtggtggtggacgtgtcccacgaggaccctgaggtgcagttcaactg
gtacgtggacggcgtggaggtgcacaacgccaagaccaagcctcgtgaggagcaattcaacagcaccttccgtgtggt
gtccgtgcttaccgtggtgcaccaagactggctgaacggcaaggagtacaagtgtaaggtgagcaacaagggacttcc
tgcccctatcgagaagaccatctccaagaccaagggccaacctcgtgagcctcaagtgtacacccttcctcctagccgtg
aggagatgaccaagaaccaagtgtcccttacctgtctggtgaagggcttctaccctagcgacatcgccgtggagtggga
gtccaacggacaacctgagaacaactacaagaccacccctcctatgcttgacagcgacggctccttcttcctgtacagca
agctgaccgtggacaagtcccgttggcaacaaggcaacgtgttcagctgttccgtgatgcacgaggccctgcacaacca
ctacacccaaaagagcctttccctgagccctggaaag

Secretory signal sequence(1-57),Variable region(58-411),Constant region(412-1389)

[Figure 36] The amino acid sequence of the hMAb1-H3-type heavy chain (SEQ ID NO: 60)

MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMS
WVRQAPGKRLEWVATISNGGTYTYYPDSVKGRFTISRDNAKNTLYLQMNSLRA
EDTAVYYCARLIYYDYLDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQT
YTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTV
VHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Secretory signal sequence(1-19), Variable region(20-137), Constant region(138-463)

[Figure 37] The nucleotide sequence of cDNA encoding a hMAb1-H4-type heavy chain (SEQ ID NO: 61)

atgaaacacctgtggttcttcctcctgctggtggcagctcccagatgggtgctgagcgaggtgcagctggtggaaagcg
gcggaggcctggtgcagcctggcggctctctgagactgagctgtgccgccagcggcttcaccttcagcacctacgccatg
agctgggtccgacaggcccctggcaagcggctggaatgggtggcaaccatcagccaaggcggcacctacacctactac
cccgacagcgtgaagggccggttcaccatcagccgggacaacgccaagaacaccctgtacctgcagatgaacagcctg
cgggccgaggacaccgccgtgtactactgcgccagactgatctactacgactacctggactactggggccagggcaccc
tggtcaccgtcagctcagccagcaccaagggcccttccgtgttccctctggccccttgtagccgttccaccagcgagtccac
cgccgcccttggctgtctggtgaaggactacttccctgagcctgtgaccgtgagctggaactccggagcccttaccagcg
gcgtgcacaccttccctgccgtgctgcagtccagcggcctttactccctgagctccgtggtgaccgtgcctagctccaactt
cggcacccaaacctacacctgtaacgtggaccacaagcctagcaacaccaaggtggacaagaccgtggagcgtaagt
gttgtgtggagtgtcctccttgtcctgcccctcctgtggccggaccttccgtgttccttttccctcctaagcctaaggacaccc
tgatgatcagccgtacccctgaggtgacctgtgtggtggtggacgtgtcccacgaggaccctgaggtgcagttcaactg
gtacgtggacggcgtggaggtgcacaacgccaagaccaagcctcgtgaggagcaattcaacagcaccttccgtgtggt
gtccgtgcttaccgtggtgcaccaagactggctgaacggcaaggagtacaagtgtaaggtgagcaacaagggacttcc
tgcccctatcgagaagaccatctccaagaccaagggccaacctcgtgagcctcaagtgtacacccttcctcctagccgtg
aggagatgaccaagaaccaagtgtcccttacctgtctggtgaagggcttctaccctagcgacatcgccgtggagtggga
gtccaacggacaacctgagaacaactacaagaccacccctcctatgcttgacagcgacggctccttcttcctgtacagca
agctgaccgtggacaagtcccgttggcaacaaggcaacgtgttcagctgttccgtgatgcacgaggccctgcacaacca
ctacacccaaaagagcctttccctgagccctggaaag

Secretory signal sequence(1-57),Variable region(58-411),Constant region(412-1389)

[Figure 38] The amino acid sequence of the hMAb1-H4-type heavy chain (SEQ ID NO: 62)

MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMS
WVRQAPGKRLEWVATISQGGTYTYYPDSVKGRFTISRDNAKNTLYLQMNSLRA
EDTAVYYCARLIYYDYLDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQT
YTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTV
VHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Secretory signal sequence(1-19), Variable region(20-137), Constant region(138-463)

[Figure 39] The nucleotide sequence of cDNA encoding a hMAb1-L1-type light chain (SEQ ID NO: 63)

atggtgctgcagacccaggtgttcatctccctgctgctgtggatctccggcgcgtacggcgacatcgtgatgacccagag
ccccctgagcctgcccgtgacactgggcgagcctgccagcatcagctgcagaagcagccagagcctggaaaacagcaa
cggcaacacctacctgaactggtatctgcagaagcccggccagtccccccagctgctgatctaccgggtgtccaaccggt
tcagcggcgtgcccgacagattcagcggcagcggctccggcaccgacttcaccctgaagatcagccgggtggaagccg
aggacgtgggcgtgtactactgtctgcaggtcacacacgtgccctggaccttcggccctggcaccaaggtggacatcaa
gcgtacggtggccgccccctccgtgttcatcttcccccctccgacgagcagctgaagtccggcaccgcctccgtggtgtgc
ctgctgaataacttctaccccagagaggccaaggtgcagtggaaggtggacaacgccctgcagtccgggaactcccag
gagagcgtgaccgagcaggacagcaaggacagcacctacagcctgagcagcaccctgaccctgagcaaagccgacta
cgagaagcacaaggtgtacgcctgcgaggtgacccaccagggcctgagctcccccgtcaccaagagcttcaacagggg
ggagtgt

Secretory signal sequence(1-60), Variable region(61-402), Constant region(403-717)

[Figure 40] The amino acid sequence of the hMAb1-L1-type light chain (SEQ ID NO: 64)

MVLQTQVFISLLLWISGAYGDIVMTQSPLSLPVTLGEPASISCRSSQSLENSNGNT
YLNWYLQKPGQSPQLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGV
YYCLQVTHVPWTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY
PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

Secretory signal sequence(1-20), Variable region(21-134), Constant region(135-239)

[Figure 41] The nucleotide sequence of cDNA encoding a hMAb1-L2-type light chain (SEQ ID NO: 65)

atggtgctgcagacccaggtgttcatctccctgctgctgtggatctccggcgcgtacggcgacatcgtgatgacccagag
ccccctgagcctgcccgtgacactgggcgagcctgccagcatcagctgcagaagcagccagagcctggaaaacgagaa
caagaacctgtacctgaactggtatctgcagaagcccggccagtccccccagctgctgatctaccgggtgtccaaccggt
tcagcggcgtgcccgacagattcagcggcagcggctccggcaccgacttcaccctgaagatcagccgggtggaagccg
aggacgtgggcgtgtactactgtctgcaggtcacacacgtgccctggaccttcggccctggcaccaaggtggacatcaa
gcgtacggtggccgcccccctccgtgttcatcttcccccccctccgacgagcagctgaagtccggcaccgcctccgtggtgtgc
ctgctgaataacttctaccccagagaggccaaggtgcagtggaaggtggacaacgccctgcagtccgggaactcccag
gagagcgtgaccgagcaggacagcaaggacagcacctacagcctgagcagcaccctgaccctgagcaaagccgacta
cgagaagcacaaggtgtacgcctgcgaggtgacccaccagggcctgagctccccgtcaccaagagcttcaacagggg
ggagtgt

Secretory signal sequence(1-60), Variable region(61-402), Constant region(403-717)

[[Figure 42] The amino acid sequence of the hMAb1-L2-type light chain (SEQ ID NO: 66)

MVLQTQVFISLLLWISGAYGDIVMTQSPLSLPVTLGEPASISCRSSQSLENENKNL
YLNWYLQKPGQSPQLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGV
YYCLQVTHVPWTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY
PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

Secretory signal sequence(1-20), Variable region(21-134), Constant region(135-239)

[Figure 43] The amino acid sequence of CDRH1 of the hMAb1-H2- or hMAb1-H4-type heavy chain (SEQ ID NO: 67).

TYAMS

[Figure 44] The amino acid sequence of CDRH2 of the hMAb1-H2- or hMAb1-H4-type heavy chain (SEQ ID NO: 68)

TISQGGTYTYYPDSVKG

[Figure 45] The amino acid sequence of CDRH3 of the hMAb1-H2- or hMAb1-H4-type heavy chain (SEQ ID NO: 69)

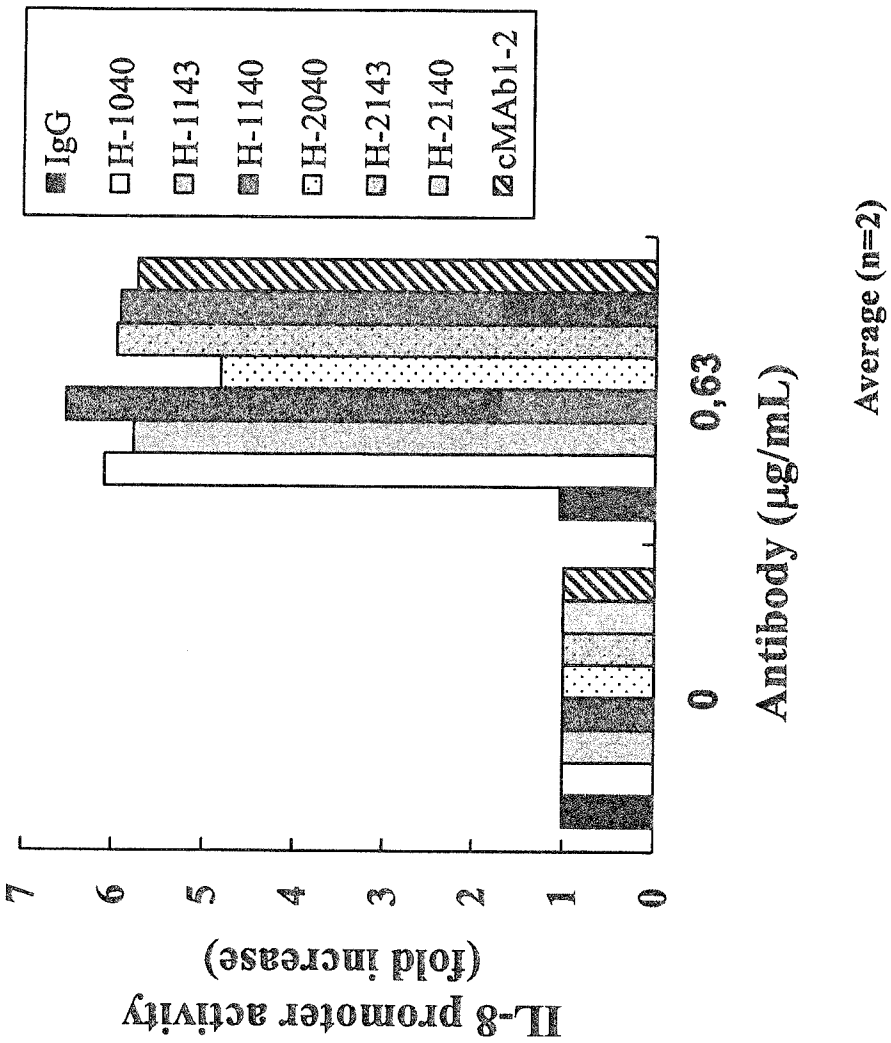
LIYYDYLDY

[Figure 46] The amino acid sequence of CDRL1 of the hMAb1-L2-type light chain (SEQ ID NO: 70)
RSSQSLENENKNLYLN

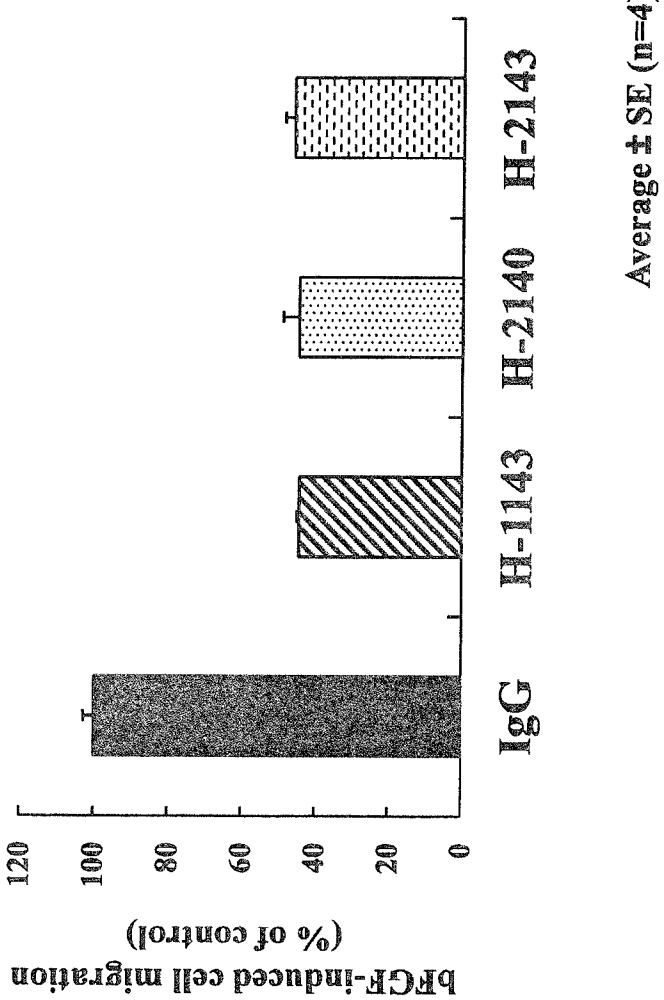
[Figure 47] The amino acid sequence of CDRL2 of the hMAb1-L2-type light chain (SEQ ID NO: 71)
RVSNRFS

[Figure 48] The amino acid sequence of CDRL3 of the hMAb1-L2-type light chain (SEQ ID NO: 72).
LQVTHVPWT

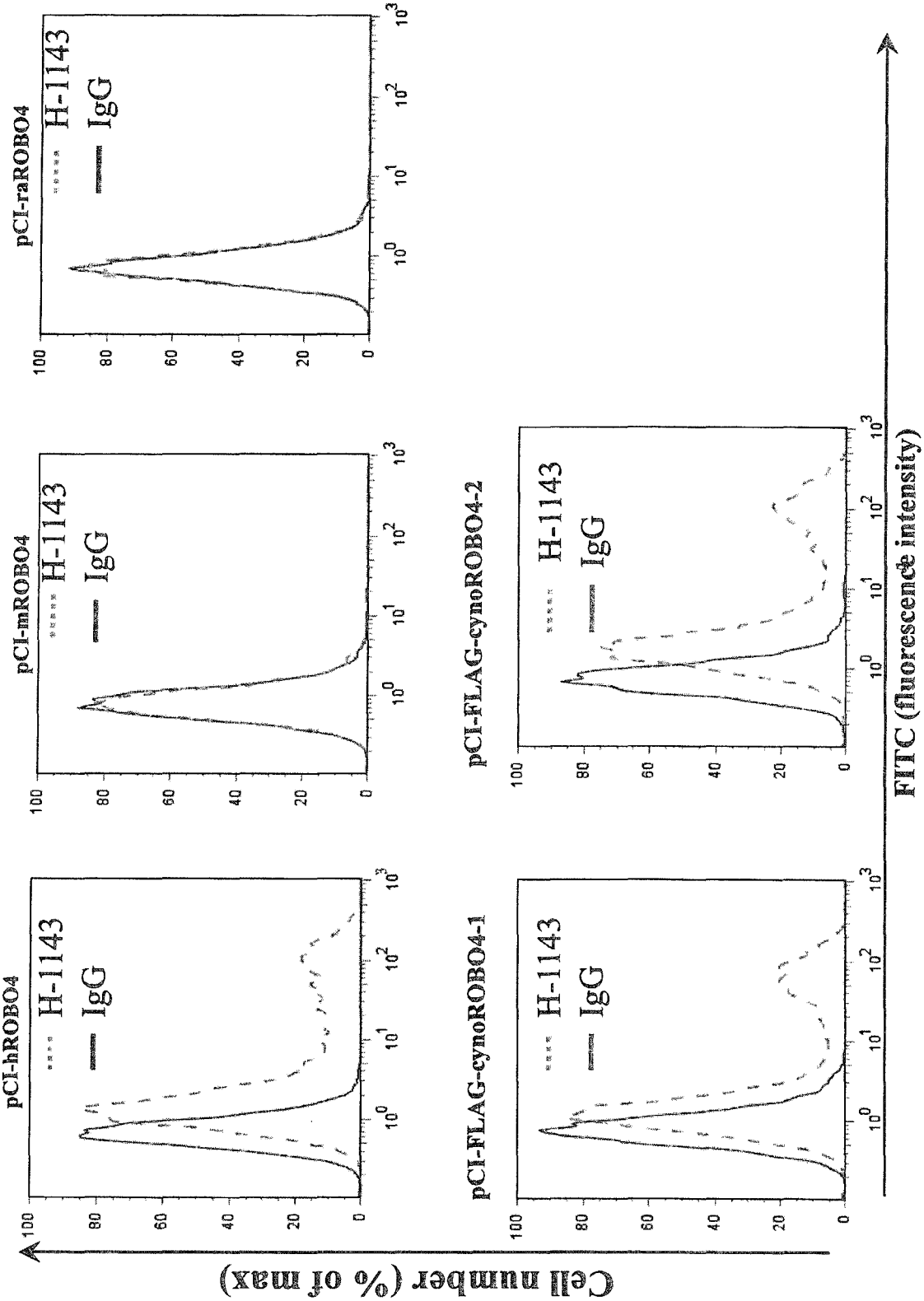
[Figure 49] IL-8 promoter activity assay



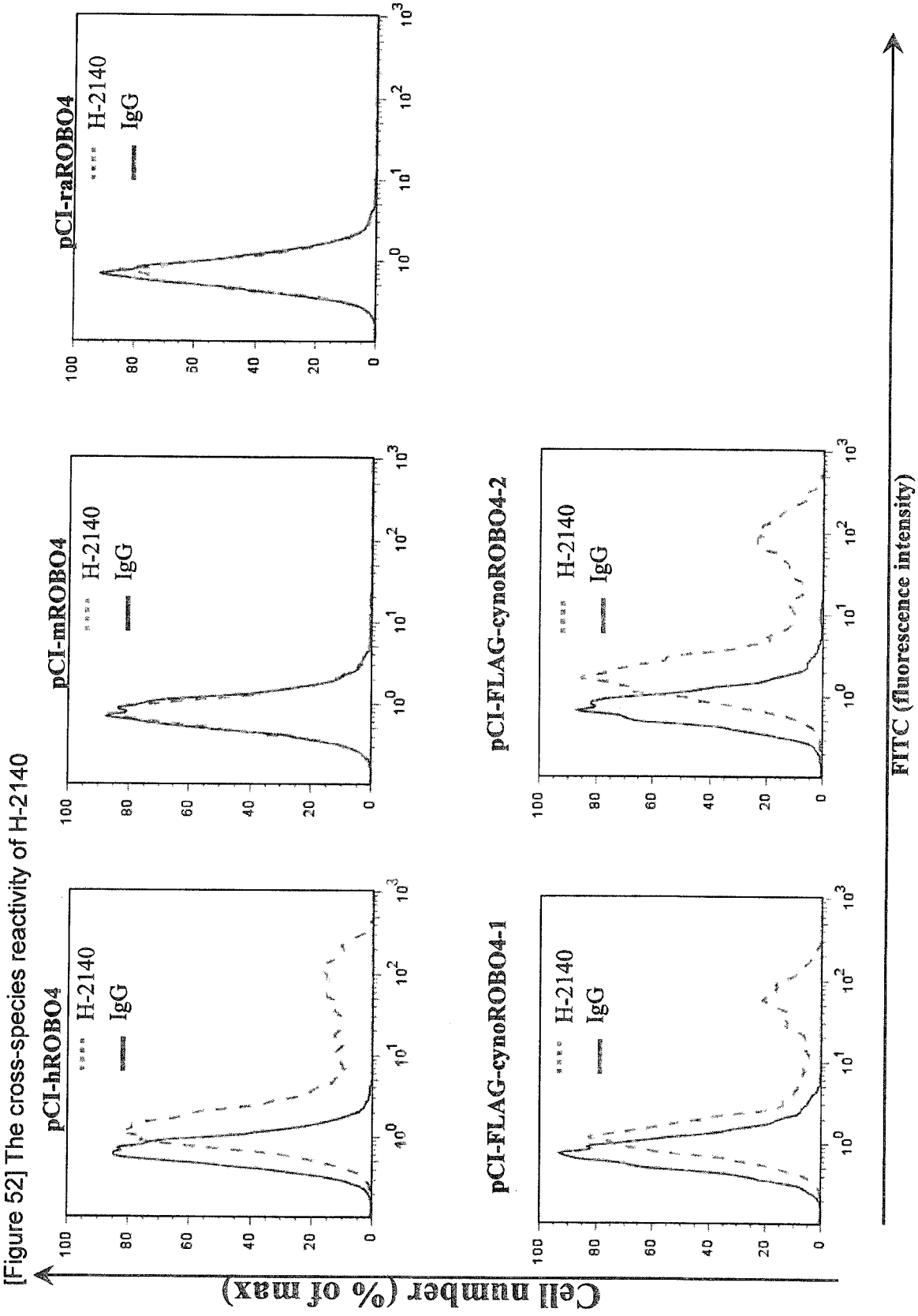
[Figure 50] bFGF-induced HUVEC migration assay



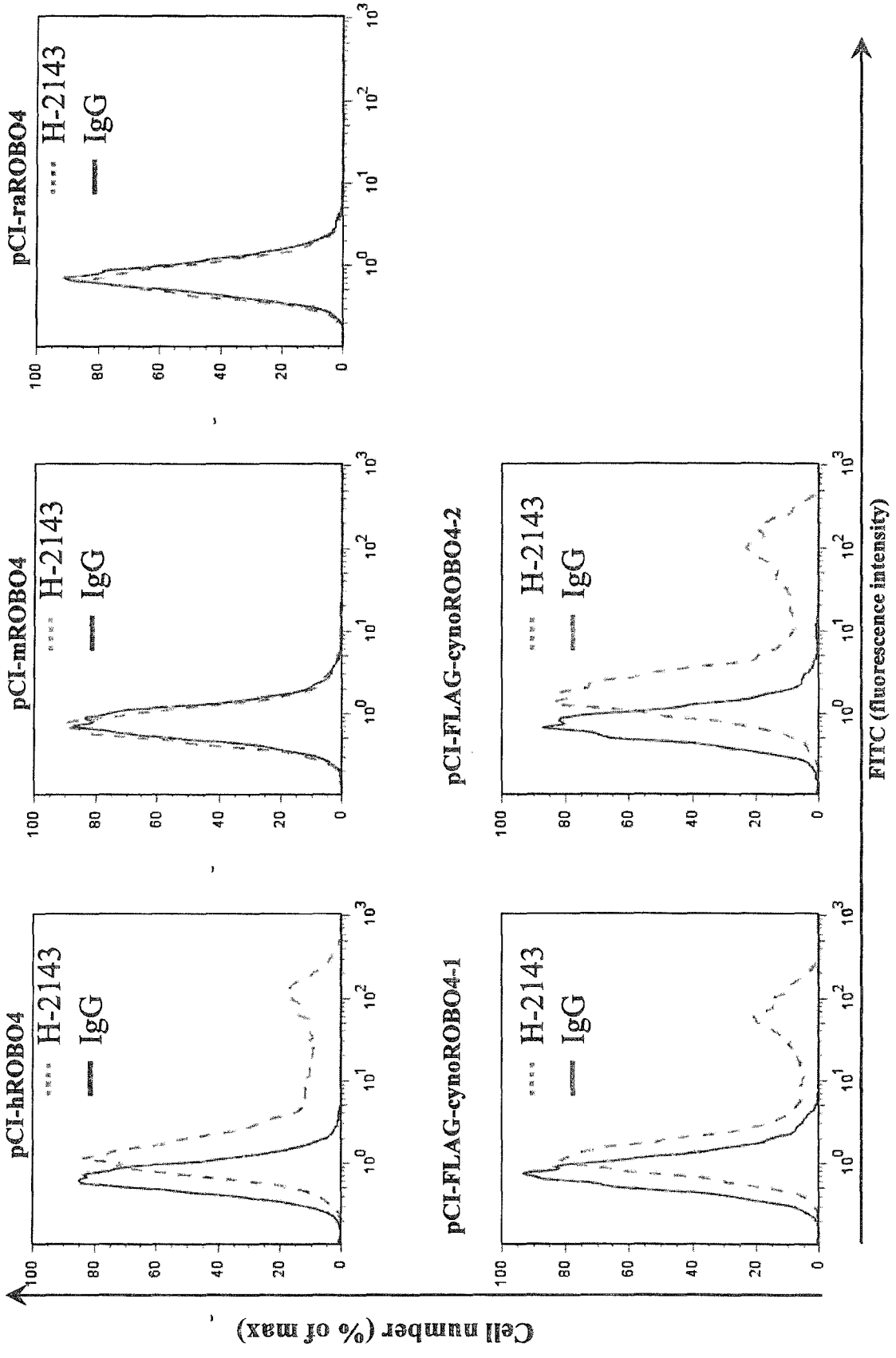
[Figure 51] The cross-species reactivity of H-1143



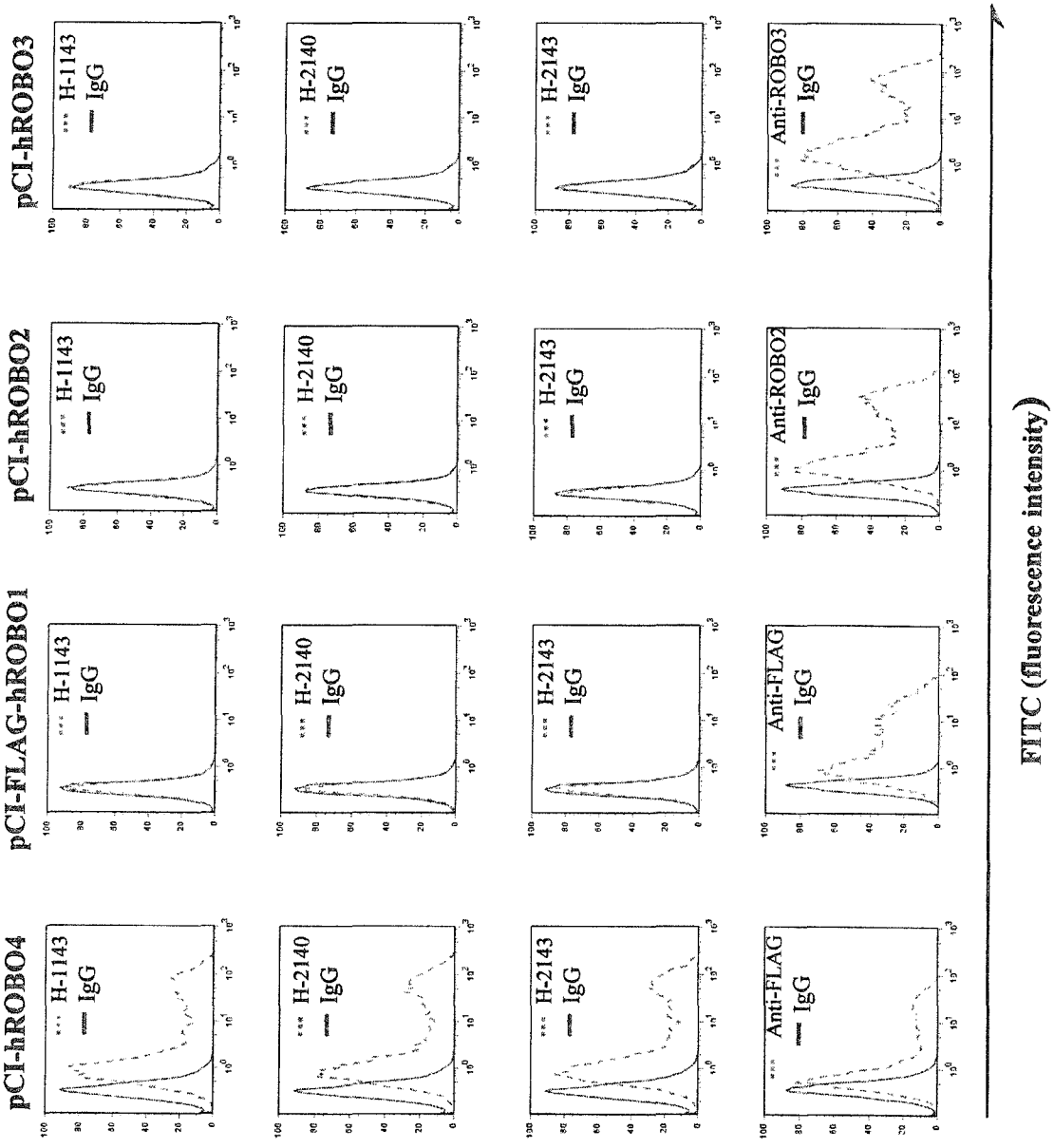
[Figure 52] The cross-species reactivity of H-2140

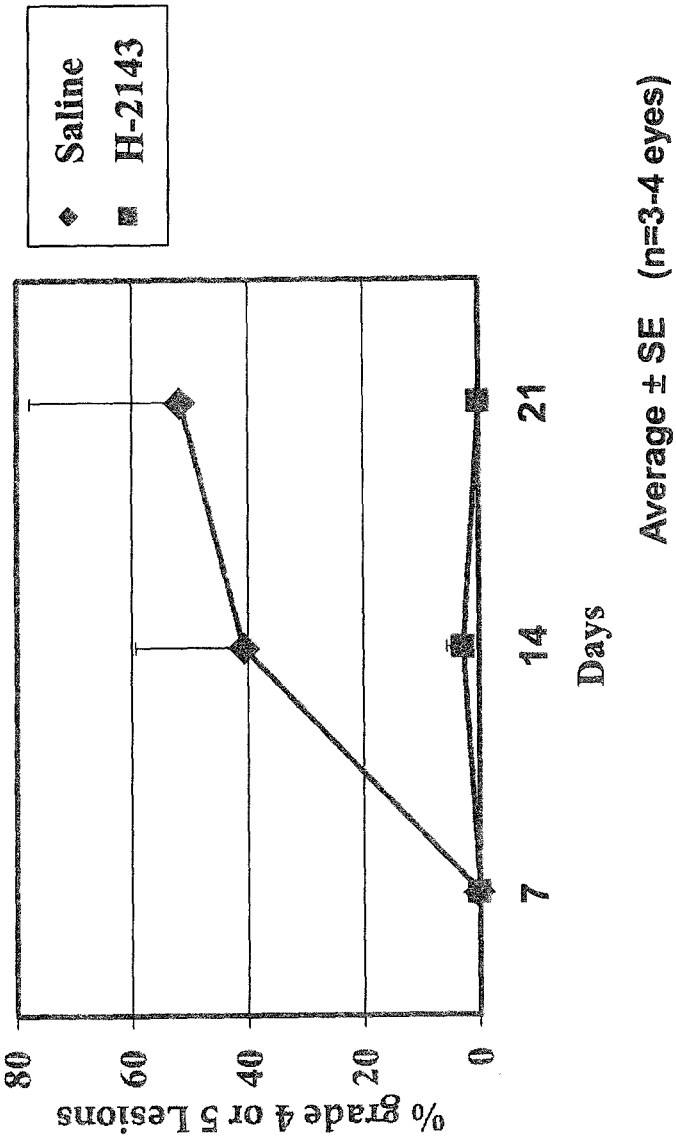


[Figure 53] The cross-species reactivity of H-2143



[Figure 54] The binding specificity of H-1143, H-2140, or H-2143





[Figure 55] In vivo efficacy of H-2143