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DESCRIPTION



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ANTI-EPHA2 ANTIBODY

Technical Field

The present invention relates to an antibody having an inhibitory activity against cell malignant transformation and/or tumor cell growth. More specifically, the present invention relates to an antibody against EPHA2 and a pharmaceutical composition comprising the antibody.

Background Art

EPHA2 is a receptor tyrosine kinase that has a molecular weight of 130 kDa and has a single transmembrane domain (Molecular and Cellular Biology, 1990, vol. 10, p. 6316-6324). EPHA2 has a ligand-binding domain and two fibronectin type 3 domains present in the N-terminal extracellular region and a tyrosine kinase domain and a sterile- α -motif (SAM) domain present in the C-terminal intracellular region.

GPI-anchored plasma membrane proteins ephrin-A1 to ephrin-A5 are known as EPHA2 ligands (Annual Review of Neuroscience, 1998, vol. 21, p. 309-345). The ligand binding to EPHA2 activates the tyrosine kinase domain and phosphorylates tyrosine residues present in the EPHA2 intracellular region, resulting in signal transduction within the cell. It has also been reported that EPHA2

bound with the ligand is internalized into the cell through endocytosis and is eventually degraded by a proteasome (Molecular Cancer Research, 2002, vol. 1, p. 79-87).

High expression of EPHA2 has been reported clinically in many cancers, particularly, breast cancer, esophagus cancer, prostate cancer, gastric cancer, non-small cell lung cancer, colon cancer, and glioblastoma multiforme (Cancer Research, 2001, vol. 61, p. 2301-2306; International Journal of Cancer, 2003, vol. 103, p. 657-663; The Prostate, 1999, vol. 41, p. 275-280; American Journal of Pathology, 2003, vol. 163, p. 2271-2276; Cancer Science, 2005, vol. 96, p. 42-47; Clinical Cancer Research, 2003, vol. 9, p. 613-618; Oncology Reports, 2004, vol. 11, p. 605-611; and Molecular Cancer Research, 2005, vol. 3, p. 541-551). It has also been reported that: for esophagus cancer, EPHA2 expression-positive patients tend to have a high frequency of regional lymph node metastasis, a large number of lymph node metastases, and a poor degree of tumor differentiation and a low five-year survival rate (International Journal of Cancer, 2003, vol. 103, p. 657-663); for non-small cell lung cancer, patients highly expressing EPHA2 tend to have a low disease-free survival rate and to have recurrence, particularly of brain metastasis (Clinical Cancer Research, 2003, vol. 9, p. 613-618); and for colon cancer, EPHA2 expression-positive patients tend to have liver metastasis, lymphatic vessel invasion, and lymph node

metastasis, and many patients with high clinical stage are EPHA2 expression-positive patients (Oncology Reports, 2004, vol. 11, p. 605-611).

Moreover, it has been reported that by the introduction of EPHA2 genes into cells, non-cancer cells acquire cancer phenotypes such as anchorage-independent growth ability, tubular morphology-forming ability on the extracellular matrix, and in vivo tumor growth ability (Cancer Research, 2001, vol. 61, p. 2301-2306), and cancer cells have enhanced invasiveness through the extracellular matrix (Biochemical and Biophysical Research Communications, 2004, vol. 320, p. 1096-1102; and Oncogene, 2004, vol. 23, p. 1448-1456). In addition, it has been reported that: the invasiveness or anchorage-independent growth of cancer cells and in vivo tumor growth are inhibited by knockdown of EPHA2 expression using siRNA (Oncogene, 2004, vol. 23, p. 1448-1456; and Cancer Research, 2002, vol. 62, p. 2840-2847); and the invasiveness, anchorage-independent growth, and tubular morphology-forming ability of cancer cells are inhibited by activating EPHA2 using fusion proteins of its ligand ephrin-A1 and a human IgG Fc region and inducing EPHA2 degradation through endocytosis (Cancer Research, 2001, vol. 61, p. 2301-2306; Molecular Cancer Research, 2005, vol. 3, p. 541-551; and Biochemical and Biophysical Research Communications, 2004, vol. 320, p. 1096-1102).

On the other hand, EPHA2 has been reported to be expressed not only in cancer cells but also within tumors

or in their surrounding blood vessels (Oncogene, 2000, vol. 19, p. 6043-6052). It has been reported that in mice, EPHA2 signals are involved in angiogenesis induced by ephrin-A1, and particularly, EPHA2 expressed in vascular endothelial cells is required for the tube formation or survival of the vascular endothelial cells (Journal of Cell Science, 2004, vol. 117, p. 2037-2049). It has also been reported that fusion proteins of an EPHA2 extracellular region and a human IgG Fc region inhibit angiogenesis in vivo and exhibit an antitumor effect (Oncogene, 2002, vol. 21, p. 7011-7026).

Monoclonal antibodies are useful not only as diagnostic drugs but also as therapeutic drugs. Monoclonal antibodies are actively used particularly in the field of cancer therapy, and monoclonal antibodies against receptor tyrosine kinases such as HER2 and EGFR or against CD20 extracellular regions are used in cancer therapy and exhibit excellent effects (The New England Journal of Medicine, 2007, vol. 357, p. 39-51; Oncogene, 2007, vol. 26, p. 3661-3678; and Oncogene, 2007, vol. 26, p. 3603-3613). The mechanisms of action of the monoclonal antibodies used in cancer therapy include apoptosis induction and inhibition of growth signals. In addition, their immunoresponse-mediated action such as ADCC or CDC is also considered to play a very important role. In actuality, it has been reported that anti-HER2 antibodies (trastuzumab) or anti-CD20 antibodies (rituximab) exhibit a much weaker antitumor effect in

xenografts of nude mice deficient in FcγRs necessary for ADCC induction than in nude mice that are not deficient in FcγRs, when these antibodies are administered thereto (Nature Medicine, 2000, vol. 6, p. 443-446). It has also been reported that anti-CD20 antibodies (rituximab) exhibit a weaker antitumor effect in mice depleted of complement by the administration of cobra venom than in mice that are not depleted of complement, when the antibody is administered thereto (Blood, 2004, vol. 103, p. 2738-2743).

For EPHA2, it has been reported that agonistic anti-EPHA2 monoclonal antibodies having an activity of inducing the phosphorylation of EPHA2 tyrosine residues and an activity of inducing EPHA2 degradation, as for the ligands, inhibit the anchorage-independent growth of a breast cancer cell line and the tubular morphology formation thereof on the extracellular matrix (Cancer Research, 2002, vol. 62, p. 2840-2847). It has also been reported that agonistic anti-EPHA2 monoclonal antibodies which recognize an epitope on EPHA2 displayed on cancer cells rather than non-cancer cells and have an activity of inducing the phosphorylation of EPHA2 tyrosine residues and an activity of inducing EPHA2 degradation exhibit an antitumor effect in vivo (Cancer Research, 2003, vol. 63, p. 7907-7912; and the pamphlet of WO 03/094859). On the other hand, Kiewlich et al. have reported that their anti-EPHA2 monoclonal antibodies had an activity of inducing the phosphorylation of EPHA2

tyrosine residues and an activity of inducing EPHA2 degradation but did not exhibit an antitumor effect in vivo (Neoplasia, 2006, vol. 8, p. 18-30).

Moreover, the pamphlet of WO 2006/084226 discloses anti-EPHA2 monoclonal antibodies LUCA19, SG5, LUCA40, and SPL1 obtained by immunizing mice with cancer cells and discloses that, among these antibodies: LUCA19 and SG5 do not influence the phosphorylation of EPHA2 tyrosine residues; LUCA40 inhibits cancer cell growth in vitro; and LUCA19, SG5, and LUCA40 are internalized into cancer cells in the presence of anti-mouse antibody labeled with toxin (saporin). The document has also reported that LUCA40 and SPL1 exhibit an antitumor effect in vivo. However, the presence or absence of the agonistic activities of these antibodies remains to be clarified.

Despite these studies, an epitope for an anti-EPHA2 antibody that exhibits an antitumor effect in vivo is still unknown. No previous document has reported that a particular amino acid sequence in an EPHA2 extracellular region is useful as an epitope for a monoclonal antibody intended for cancer therapy.

Even antibodies against the same antigen differ in their properties depending on differences in epitopes or their sequences. Furthermore, due to this difference in their properties, the antibodies, when administered to humans, would clinically respond in different a manner with respect to drug effectiveness, the frequency of

therapeutic response, side effects, the frequency of occurrence of drug resistance, etc.

Thus, a drug having clinically the best properties may also differ depending on the patient. In many cases, such properties are unknown until the drug is actually administered. Thus, it has been strongly required to develop a drug having a novel mechanism of action. It has also been strongly required to develop an antibody against EPHA2 having properties different from those of conventional antibodies.

Disclosure of the Invention

Problems to be Solved by the Invention

An object of the present invention is to provide an antibody against EPHA2.

A further object of the present invention is to provide a pharmaceutical composition and the like comprising an anti-EPHA2 antibody having a therapeutic effect on cancer.

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 inhibiting tumor growth using the antibody, etc.

Means for Solving the Problems

The present inventors have conducted diligent studies to attain the objects and consequently

successfully obtained a novel anti-EPHA2 monoclonal antibody which has no activity of inducing the phosphorylation of EPHA2 tyrosine residues and has ADCC and CDC activities against EPHA2-expressing cancer cells. Furthermore, the present inventors have examined an epitope for this antibody and consequently found for the first time that an antibody that binds to a region comprising, of two fibronectin type 3 domains present in EPHA2, the c-terminal domain, has an excellent antitumor activity in vivo. Based on these findings, the present invention has been completed.

Specifically, the present invention comprises:

- (1) An antibody which recognizes an epitope recognized by an antibody produced by the hybridoma SH348-1 (FERM BP-10836);
- (2) The antibody according to (1), wherein the antibody has the following properties a) to d):
 - a) having no ability to phosphorylate EPHA2 tyrosine residues;
 - b) having an ADCC activity against EPHA2-expressing cells;
 - c) having a CDC activity against EPHA2-expressing cells; and
 - d) having an antitumor activity in vivo;
- (3) The antibody according to (1), wherein the antibody has the following properties a) to e):
 - a) having no ability to phosphorylate EPHA2 tyrosine residues;

b) exhibiting an effect of decreasing an EPHA2 protein level;

c) having an ADCC activity against EPHA2-expressing cells;

d) having a CDC activity against EPHA2-expressing cells; and

e) having an antitumor activity in vivo;

(4) An antibody which specifically binds to a polypeptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing;

(5) An antibody which specifically binds to a polypeptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing and has the following properties a) to d):

a) having no ability to phosphorylate EPHA2 tyrosine residues;

b) having an ADCC activity against EPHA2-expressing cells;

c) having a CDC activity against EPHA2-expressing cells; and

d) having an antitumor activity in vivo;

(6) An antibody which specifically binds to a peptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing and has the following properties a) to e):

a) having no ability to phosphorylate EPHA2 tyrosine residues;

b) exhibiting an effect of decreasing an EPHA2 protein level;

c) having an ADCC activity against EPHA2-expressing cells;

d) having a CDC activity against EPHA2-expressing cells; and

e) having an antitumor activity in vivo;

(7) The antibody according to any one of (1) to (6), wherein the antibody specifically binds to a peptide consisting of an amino acid sequence represented by amino acid Nos. 439 to 534 of SEQ ID NO: 8 in the sequence listing;

(8) The antibody according to any one of (1) to (7), wherein the antibody inhibits the phosphorylation of EPHA2 tyrosine residues induced by an EPHA2 ligand;

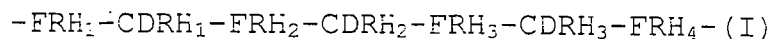
(9) The antibody according to any one of (1) to (7), wherein the antibody does not inhibit EPHA2 ligand binding to EPHA2 but inhibits the phosphorylation of EPHA2 tyrosine residues induced by the ligand;

(10) An antibody which specifically binds to a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 8 in the sequence listing, wherein the antibody has the amino acid sequences represented by SEQ ID NOs: 59, 61, and 63 in the sequence listing, or amino acid sequences having deletion, substitution, or addition of one or more amino acids in

the amino acid sequences, as the complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOs: 65, 67, and 69 in the sequence listing, or amino acid sequences having deletion, substitution, or addition of one or more amino acids in the amino acid sequences, as complementarity determining regions in the light chain variable region;

(11) An antibody which specifically binds to a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 8 in the sequence listing, characterized by the following 1) and 2):

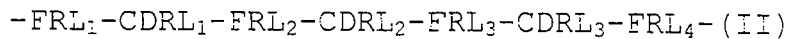
1) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):



wherein FRH_1 represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids; $CDRH_1$ represents the amino acid sequence represented by SEQ ID NO: 59 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRH_2 represents an arbitrary amino acid sequence consisting of 14 amino acids; $CDRH_2$ represents the amino acid sequence represented by SEQ ID NO: 61 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRH_3 represents an arbitrary amino acid sequence consisting of 32 amino acids; $CDRH_3$ represents

the amino acid sequence represented by SEQ ID NO: 63 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; and FRH₄ represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and

2) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):



wherein FRL₁ represents an arbitrary amino acid sequence consisting of 23 amino acids; CDRL₁ represents the amino acid sequence represented by SEQ ID NO: 65 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRL₂ represents an arbitrary amino acid sequence consisting of 15 amino acids; CDRL₂ represents the amino acid sequence represented by SEQ ID NO: 67 in the sequence listing; FRL₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRL₃ represents the amino acid sequence represented by SEQ ID NO: 69 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; and FRL₄ represents an arbitrary amino acid sequence consisting of 10 amino

acids, wherein these amino acids are linked to each other through peptide bonds;

(12) An antibody which recognizes an epitope recognized by an antibody produced by the hybridoma SH357-1 (FERM BP-10837);

(13) The antibody according to (12), wherein the antibody has the following properties a) to d):

a) having no ability to phosphorylate EPHA2 tyrosine residues;

b) having an ADCC activity against EPHA2-expressing cells;

c) having a CDC activity against EPHA2-expressing cells; and

d) having an antitumor activity in vivo;

(14) An antibody which specifically binds to a peptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing and has the following properties a) to e):

a) having no ability to phosphorylate EPHA2 tyrosine residues;

b) exhibiting no effect of decreasing an EPHA2 protein level;

c) having an ADCC activity against EPHA2-expressing cells;

d) having a CDC activity against EPHA2-expressing cells; and

e) having an antitumor activity in vivo;

(15) The antibody according to any one of (12) to (14), wherein the antibody specifically binds to a peptide consisting of an amino acid sequence represented by amino acid Nos. 439 to 534 of SEQ ID NO: 8 in the sequence listing;

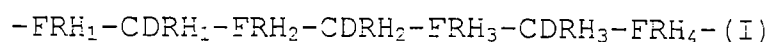
(16) The antibody according to any one of (12) to (15), wherein the antibody inhibits the phosphorylation of EPHA2 tyrosine residues induced by an EPHA2 ligand;

(17) The antibody according to any one of (12) to (15), wherein the antibody does not inhibit EPHA2 ligand binding to EPHA2 but inhibits the phosphorylation of EPHA2 tyrosine residues induced by the ligand;

(18) An antibody which specifically binds to a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 8 in the sequence listing, wherein the antibody has the amino acid sequences represented by SEQ ID NOs: 71, 73, and 75 in the sequence listing, or amino acid sequences having deletion, substitution, or addition of one or more amino acids in the amino acid sequences, as complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOs: 77, 79, and 81 in the sequence listing, or amino acid sequences having deletion, substitution, or addition of one or more amino acids in the amino acid sequences, as complementarity determining regions in the light chain variable region;

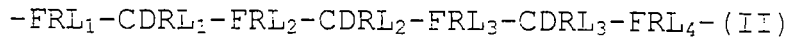
(19) An antibody which specifically binds to a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 8 in the sequence listing, characterized by the following 1) and 2):

1) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):



wherein FRH_1 represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids; $CDRH_1$ represents the amino acid sequence represented by SEQ ID NO: 71 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRH_2 represents an arbitrary amino acid sequence consisting of 14 amino acids; $CDRH_2$ represents the amino acid sequence represented by SEQ ID NO: 73 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRH_3 represents an arbitrary amino acid sequence consisting of 32 amino acids; $CDRH_3$ represents the amino acid sequence represented by SEQ ID NO: 75 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; and FRH_4 represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and

2) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):



wherein FRL_1 represents an arbitrary amino acid sequence consisting of 23 amino acids; $CDRL_1$ represents the amino acid sequence represented by SEQ ID NO: 77 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRL_2 represents an arbitrary amino acid sequence consisting of 15 amino acids; $CDRL_2$ represents the amino acid sequence represented by SEQ ID NO: 79 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRL_3 represents an arbitrary amino acid sequence consisting of 32 amino acids; $CDRL_3$ represents the amino acid sequence represented by SEQ ID NO: 81 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; and FRL_4 represents an arbitrary amino acid sequence consisting of 10 amino acids, wherein these amino acids are linked to each other through peptide bonds;

(20) The antibody according to any one of (1) to (19), characterized in that the antibody is a humanized antibody;

(21) The antibody according to any one of (1) to (19), characterized in that the antibody is a human antibody;

(22) The antibody according to any one of (1) to (19), characterized in that the antibody is an IgG antibody;

(23) The antibody according to any one of (1) to (9) and (12) to (17), characterized in that the antibody is any selected from Fab, F(ab')₂, Fv, scFv, a diabody, a linear antibody, and a multispecific antibody;

(24) An antibody produced by the hybridoma SH348-1 (FERM BP-10836);

(25) An antibody produced by the hybridoma SH357-1 (FERM BP-10837);

(26) An antibody consisting of the following 1) and 2):

1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 35 in the sequence listing or a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 39 in the sequence listing; and

2) a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 37 in the sequence listing or a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 41 in the sequence listing;

(27) An antibody consisting of the following 1) or 2):

1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of

SEQ ID NO: 35 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 37 in the sequence listing; and

2) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 39 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 41 in the sequence listing;

(28) An antibody consisting of the following 1) and 2):

1) a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 35 in the sequence listing or a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 39 in the sequence listing; and

2) a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 37 in the sequence listing or a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 41 in the sequence listing;

(29) An antibody consisting of the following 1) or 2):

1) a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 35 in the sequence listing and a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 37 in the sequence listing; and

2) a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 39 in the sequence listing and a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 41 in the sequence listing;

(30) An antibody obtained by humanizing an antibody according to any one of (24) to (29);

(31) An antibody consisting of the following 1) and 2):

1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 107 in the sequence listing or a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 115 in the sequence listing; and

2) a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 91 in the sequence listing or a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 99 in the sequence listing;

(32) An antibody consisting of the following 1) or 2):

1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 107 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 91 in the sequence listing; and

2) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 115 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 99 in the sequence listing;

(33) An antibody consisting of the following 1) and 2):

1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 139 in the sequence listing or a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 147 in the sequence listing; and

2) a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 123 in the sequence listing or a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 131 in the sequence listing;

(34) An antibody consisting of the following 1) or 2):

1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 139 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 123 in the sequence listing; and

2) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of

SEQ ID NO: 147 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 131 in the sequence listing;

(35) A Fab, F(ab')₂, Fv, scFv, a diabody, a linear antibody, or a multispecific antibody derived from antibodies according to any of (24) to (34);

(36) Any one polypeptide selected from the group consisting of the following 1) to 20):

1) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 35 in the sequence listing;

2) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 39 in the sequence listing;

3) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 37 in the sequence listing;

4) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 41 in the sequence listing;

5) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 107 in the sequence listing;

6) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 115 in the sequence listing;

7) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 107 in the sequence listing;

8) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 115 in the sequence listing;

9) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 91 in the sequence listing;

10) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 99 in the sequence listing;

11) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 91 in the sequence listing;

12) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 99 in the sequence listing;

13) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 139 in the sequence listing;

14) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 147 in the sequence listing;

15) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 139 in the sequence listing;

16) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 147 in the sequence listing;

17) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 123 in the sequence listing;

18) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 131 in the sequence listing;

19) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 123 in the sequence listing; and

20) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 131 in the sequence listing;

(37) The mouse hybridoma SH-348-1 (FERM BP-10836);

(38) The mouse hybridoma SH-357-1 (FERM BP-10837);

(39) A pharmaceutical composition characterized by comprising at least one antibody selected from the antibodies according to (1) to (36);

(40) A pharmaceutical composition for cancer treatment characterized by comprising at least one antibody selected from the antibodies according to (1) to (36);

(41) A method for inhibiting tumor growth in a mammal, comprising administering any antibody selected from the group consisting of antibodies according to (1) to (36), (39), and (40);

- (42) The method for inhibiting tumor growth according to (41), characterized in that the tumor is a tumor expressing EPHA2;
- (43) A polynucleotide encoding an antibody according to any one of (1) to (36);
- (44) A host cell transformed with a polynucleotide according to (43); and
- (45) A method for producing an antibody using a host cell according to (44).

Advantages of the Invention

According to the present invention, a novel anti-EPHA2 monoclonal antibody has been successfully obtained, which has no activity of inducing the phosphorylation of EPHA2 tyrosine residues and has ADCC and CDC activities against EPHA2-expressing cancer cells. Furthermore, it has been found that the antibody has excellent antitumor activity in vivo.

Furthermore, a pharmaceutical composition for cancer treatment comprising the antibody has been provided.

Brief Description of the Drawings

Figure 1 is a diagram showing Western blotting results showing the presence or absence of the activity of inducing the phosphorylation of EPHA2 tyrosine residues by an anti-EPHA2 antibody. Figure 1A) is a diagram showing the results obtained in the absence of a cross-linking antibody, wherein the upper bar shows the

results for a 4G10 antibody, and the lower bar shows the results for an anti-EPHA2 antibody (D7). Figure 1B) is a diagram showing the results obtained in the presence of a cross-linking antibody, wherein the upper bar shows the results of a 4G10 antibody, and the lower bar shows the results of an anti-EPHA2 antibody (D7);

Figure 2 is a diagram showing Western blotting results showing the presence or absence of the activity of inducing a decrease in EPHA2 protein level by an anti-EPHA2 antibody. Figure 2A) is a diagram showing the results obtained in the absence of a cross-linking antibody, wherein the upper bar shows the results for an anti-EPHA2 antibody (D7), and the lower bar shows the results for an anti-b-actin antibody. Figure 2B) is a diagram showing the results obtained in the presence of a cross-linking antibody, wherein the upper bar shows the results for an anti-EPHA2 antibody (D7), and the lower bar shows the results for an anti-b-actin antibody;

Figure 3 is a diagram showing the presence or absence of the ADCC activity of an anti-EPHA2 antibody against various cell lines. In the diagram, "***" represents $P < 0.01$, and "****" represents $P < 0.001$. Figure 3A) is a diagram showing the ADCC activity against MDA-MB-231 cells. Figure 3B) is a diagram showing the ADCC activity against A549 cells. Figure 3C) is a diagram showing the ADCC activity against PC-3 cells;

Figure 4 is a diagram showing the presence or absence of the CDC activity of an anti-EPHA2 antibody

against various cells. In the diagram, "****" represents $P < 0.001$. Figure 4A) is a diagram showing the CDC activity of SH348-1 against MDA-MB-231 cells. Figure 4B) is a diagram showing the CDC activity of SH348-1 against A549 cells. Figure 4C) is a diagram showing the CDC activity of SH348-1 against PC-3 cells. Figure 4D) is a diagram showing the CDC activity of SH357-1 against MDA-MB-231 cells. Figure 4E) is a diagram showing the CDC activity of SH357-1 against A549 cells. Figure 4F) is a diagram showing the CDC activity of SH357-1 against PC-3 cells;

Figure 5A) is a diagram showing EPHA2 domain structure prediction and the positions, in EPHA2, of EPHA2-ECD, FNIII-NC, FNIII-N, and FNIII-C which are peptides for epitope determination. In the diagram, Ligand-BD represents a ligand-binding domain, FN3 represents a fibronectin type 3 domain, TM represents a transmembrane region, Trk kinase represents a tyrosine kinase domain, and SAM represents a SAM domain;

Figure 5B) is a diagram showing the presence or absence of the binding activity of an anti-EPHA2 antibody for EPHA2-ECD, FNIII-NC, FNIII-N, and FNIII-C;

Figure 6A) is a diagram showing the antitumor activity of SH348-1 against MDA-MB-231 cell-transplanted mice;

Figure 6B) is a diagram showing the antitumor activity of SH357-1 against MDA-MB-231 cell-transplanted

mice. In the diagram, the error bar represents a standard error ($n = 9$);

Figure 7A) is a diagram showing the binding activity of SH348-1 to an EPHA2 extracellular region polypeptide;

Figure 7B) is a diagram showing the binding activity of SH357-1 to an EPHA2 extracellular region polypeptide;

Figure 7C) is a diagram showing the binding activity of Ab96-1 to an EPHA2 extracellular region polypeptide;

Figure 8 is a diagram showing the ligand binding inhibitory activities of SH348-1, SH357-1, and Ab96-1;

Figure 9 is a diagram showing that SH348-1 and SH357-1 have an activity of inhibiting the ephrin-A1-dependent phosphorylation of EPHA2 tyrosine residues;

Figure 10 is a diagram showing the outline of deletion mutants of EPHA2 for epitope identification;

Figure 11A) is a diagram showing the reactivity of SH348-1 to deletion mutants of EPHA2;

Figure 11B) is a diagram showing the detection of the deletion mutants of EPHA2 on a PVDF membrane in Figure 11A);

Figure 11C) is a diagram showing the reactivity of SH357-1 to deletion mutants of EPHA2;

Figure 11D) is a diagram showing the detection of the deletion mutants of EPHA2 on a PVDF membrane in Figure 11C);

Figure 12A) is a diagram showing the binding activity of hSH348-1-T1 to an EPHA2 extracellular region polypeptide;

Figure 12B) is a diagram showing the binding activity of hSH348-1-T3 to an EPHA2 extracellular region polypeptide;

Figure 12C) is a diagram showing the binding activity of hSH357-1-T1 to an EPHA2 extracellular region polypeptide;

Figure 12D) is a diagram showing the binding activity of hSH357-1-T3 to an EPHA2 extracellular region polypeptide;

Figure 13A) is a diagram showing the competitive inhibitory activities of hSH348-1-T1 and hSH348-1-T3 against the antigen binding of SH348-1;

Figure 13B) is a diagram showing the competitive inhibitory activities of hSH357-1-T1 and hSH357-1-T3 against the antigen binding of SH357-1;

Figure 14A) is a diagram showing the activity of inhibiting the ephrin-A1-dependent phosphorylation of EPHA2 tyrosine residues by hSH348-1-T1 or hSH348-1-T3; and

Figure 14B) is a diagram showing the activity of inhibiting the ephrin-A1-dependent phosphorylation of EPHA2 tyrosine residues by hSH357-1-T1 or hSH357-1-T3.

Best Mode for Carrying Out the Invention

1. Definitions

In the present specification, the terms "cancer" and "tumor" are used in the same sense.

In the present specification, the term "gene" is meant to encompass not only DNA but also mRNA thereof, cDNA, and cRNA thereof. Thus, the term "EPHA2 gene" in the present invention encompasses EPHA2 DNA, mRNA, cDNA, and cRNA.

In the present specification, the term "polynucleotide" is used in the same sense as a nucleic acid and also encompasses DNA, RNA, probes, oligonucleotides, and primers.

In the present specification, the terms "polypeptide" and "protein" are used without being differentiated therebetween.

In the present specification, the term "cell" also encompasses cells in individual animal and cultured cells.

In the present specification, the term "cell malignant transformation" means that cells exhibit abnormal growth, for example, lose sensitivity to contact inhibition or exhibit anchorage-independent growth. Cells exhibiting such abnormal growth are referred to as "cancer cells".

In the present specification, a protein having equivalent functions to the cell malignant transformation activity and/or cell growth activity or the like of EPHA2 is also referred to as EPHA2.

In the present specification, the term "phosphorylation of tyrosine residues" means that tyrosine residues contained in the amino acid sequence of a peptide are phosphorylated. Whether or not tyrosine

residues are phosphorylated can be examined, for example, based on the affinity of the peptide for an anti-phosphotyrosine antibody (e.g., Anti-Phosphotyrosine, recombinant 4G10 HRP-conjugate (manufactured by Millipore (Upstate), #16-184)). The tyrosine residues can be determined to be phosphorylated, when the peptide binds to the antibody.

In the present specification, the term "ability to phosphorylate EPHA2 tyrosine residues" refers to an ability to phosphorylate tyrosine residues in the amino acid sequence of EPHA2. Whether or not an antibody has the ability to phosphorylate EPHA2 tyrosine residues can be determined, for example, by incubating the antibody and EPHA2 and then examining the presence or absence of the affinity of the EPHA2 for an anti-phosphotyrosine antibody.

In the present specification, the phrase "decreasing an EPHA2 protein expression level" means that an EPHA2 protein level is decreased. Whether or not an antibody has an effect of decreasing an EPHA2 protein level can be examined, for example, by incubating the antibody and EPHA2 and then quantifying the EPHA2 level.

In the present specification, the term "EPHA2 ligand" refers to a substance capable of serving as an EPHA2 ligand. Specific examples thereof can include GPI-anchored plasma membrane proteins ephrin-A1 to ephrin-A5 (Annual Review of Neuroscience, 1998, vol. 21, p. 309-345).

In the present specification, the term "cytotoxicity" refers to any pathologic change in cells and refers not only to direct injury but also to any structural or functional damage to cells such as DNA cleavage, dimerization of bases, chromosomal breakage, damage of mitotic apparatus, and decrease in various enzyme activities.

In the present specification, the term "cytotoxic activity" refers to an activity that causes the cytotoxicity.

In the present specification, ADCC is synonymous with antibody-dependent cellular cytotoxicity and refers to a reaction through which Fc γ receptor-bearing cells adhere via the Fc γ receptors to the Fc portions of antibodies bound with surface antigens in target cells and kill the target cells. An ADCC activity is also referred to as an antibody-dependent cytotoxic activity and refers to an activity that causes the reaction. The ADCC activity can be measured by methods usually performed by those skilled in the art and can be measured, for example, according to a method described in paragraph 3)-2 of Example 3 in the present specification.

In the present specification, the term "CDC" is synonymous with complement-dependent cytotoxicity. A CDC activity refers to an activity that causes complement-dependent cytotoxicity. The CDC activity can be measured by methods usually performed by those skilled in the art and can be measured, for example, according to a method

described in paragraph 3)-3 of Example 3 in the present specification.

In the present specification, the phrase "having an antitumor activity in vivo" means having the activity of inhibiting or reducing tumor growth in tumor-bearing animal individuals. Whether or not an anti-EPA2 antibody "has an antitumor activity in vivo" can be examined by methods usually performed by those skilled in the art and can also be examined, for example, according to the following method: an appropriate dose of the anti-EPA2 antibody is intraperitoneally administered as a test substance to tumor cell (e.g., MDA-MB-231 cell)-subcutaneously transplanted nude mice (e.g., BALB/cAJcl-nu/nu; obtained from CLEA Japan, Inc.), and the time-dependent change in tumor volume is compared between the nude mice and anti-EPA2 antibody-unadministered controls. The anti-EPA2 antibody as a test substance can be determined to "have an antitumor activity in vivo", when the tumor volume is significantly smaller in the mice than in the controls.

Each of the heavy and light chains of an antibody molecule is known to have three complementarity determining regions (CDRs). In the present specification, the complementarity determining regions of an antibody are represented by CDRH₁, CDRH₂, and CDRH₃ for the complementarity determining regions of the heavy chain and by CDRL₁, CDRL₂, and CDRL₃ for the complementarity determining regions of the light chain.

In the present specification, the term "epitope" means an EPHA2 partial peptide having antigenicity and/or immunogenicity in vivo in animals, preferably, mammals, more preferably mice or humans. The epitope as an EPHA2 partial peptide having antigenicity can be determined by methods well known by those skilled in the art, such as by an immunoassay, and can be determined, for example, according to the following method in which various partial structures of EPHA2 are prepared. For the preparation of the partial structures, oligopeptide synthesis techniques known in the art can be used. For example, a series of sequentially shorter polypeptides of appropriate lengths from the C- or N-terminus of EPHA2 is prepared using a gene recombination technique well known by those skilled in the art. Then, the reactivity of the antibody to these polypeptides is studied. A recognition site is roughly determined, and shorter peptides are then synthesized. The reactivity to these peptides can be studied to thereby determine the epitope.

1. Regarding EPHA2

(1) EPHA2 gene

The nucleotide sequence of the EPHA2 gene and the amino acid sequence thereof are recorded as EPH receptor A2 in GenBank (Accession Nos: NM_004431 and NP_004422, respectively). Moreover, the nucleotide sequence of an open reading frame (ORF) in the EPHA2 gene is described in SEQ ID NO: 1 in the sequence listing. The amino acid

sequence thereof is described in SEQ ID NO: 2 in the sequence listing.

In this context, EPHA2 also encompasses proteins which consist of an amino acid sequence derived from the EPHA2 amino acid sequence by the substitution, deletion, or addition of one or more amino acids and have an equivalent biological activity to that of this enzyme.

(2) Cancer site-specific expression of EPHA2 gene

The EPHA2 gene has been reported to be highly expressed in many cancers, particularly, breast cancer, esophagus cancer, prostate cancer, gastric cancer, non-small cell lung cancer, colon cancer, and glioblastoma multiforme.

Specifically, the expression level of EPHA2 in each cell and/or each tissue can be measured to thereby determine the state of malignant transformation and/or cancer cell growth that can be attributed to EPHA2 over-expression in test subjects.

Moreover, a substance that inhibits the expression level and/or activity of EPHA2 has an activity of inhibiting cell malignant transformation and/or cancer cell growth attributed to EPHA2.

Thus, test substances are contacted with EPHA2-expressing cells, and a substance that inhibits the expression level and/or activity of EPHA2 can be selected to thereby screen for an antitumor substance.

In this context, siRNA against EPHA2 inhibits EPHA2 expression and can be used as an antitumor agent. The

siRNA against EPHA2 can be produced by: designing, based on the nucleotide sequence of EPHA2 mRNA, RNA consisting of a partial sequence of EPHA2 mRNA (sense RNA) and RNA consisting of a nucleotide sequence complementary to the nucleotide sequence of the RNA (antisense RNA); synthesizing the RNAs by a chemical synthesis method known per se in the art; and hybridizing both the obtained RNAs. It is preferred that a sequence of one or more nucleotides called an overhang sequence should be bound to the 3'-end of each of the sense and antisense RNAs constituting the siRNA. The overhang sequence is not particularly limited as long as it protects the RNA from nuclease. Any sequence of preferably 1 to 10, more preferably 1 to 4, even more preferably 2 nucleotides can be used.

2. Antibody against EPHA2

(1) Preparation of antigen

Examples of antigens for obtaining the antibody of the present invention against EPHA2 can include a full-length polypeptide of EPHA2 and partial polypeptides thereof and can more specifically include a full-length polypeptide of EPHA2 and preferably an EPHA2 extracellular region polypeptide (consisting of an amino acid sequence represented by amino acid Nos. 1 to 534 of SEQ ID NO: 8 in the sequence listing), more preferably a partial polypeptide of the EPHA2 extracellular region polypeptide comprising an amino acid sequence represented

by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing, even more preferably a partial polypeptide of the EPHA2 extracellular region polypeptide comprising an amino acid sequence represented by amino acid Nos. 439 to 534 of SEQ ID NO: 8 in the sequence listing, and derivatives obtained by adding an arbitrary amino acid sequence or a carrier to these sequences. Further examples thereof can include polypeptides consisting of consecutive partial amino acid sequences of at least 6 amino acids and derivatives obtained by adding an arbitrary amino acid sequence or a carrier to these sequences.

In this context, the EPHA2 full-length polypeptide or the partial polypeptides thereof used as an antigen can be obtained by causing the EPHA2 gene or genes of the partial polypeptides to be expressed in host cells by genetic engineering.

EPHA2 can be directly purified, for use, from human tumor tissues or tumor cells. Moreover, the EPHA2 full-length polypeptide or the partial polypeptides thereof can be synthesized in vitro or obtained by causing host cells by genetic engineering to produce the polypeptide.

In the genetic engineering, specifically, genes encoding EPHA2 or partial polypeptides thereof are incorporated into vectors capable of expressing the EPHA2 or partial polypeptides thereof, and the EPHA2 or partial polypeptides thereof can then be synthesized in a solution containing enzymes, substrates, and energy

substances necessary for transcription and translation. Alternatively, host cells of other prokaryotes or eukaryotes can be transformed therewith and caused to express the EPHA2 or partial polypeptides thereof to obtain the desired protein.

cDNA of the partial polypeptide of EPHA2 can be obtained, for example, by a so-called polymerase chain reaction (hereinafter, referred to as "PCR") method in which PCR (see Saiki, R.K., et al. Science (1988) 239, p. 487-489) is performed using EPHA2-expressing cDNA libraries as templates and primers specifically amplifying EPHA2 cDNA or DNA encoding the partial polypeptide.

Examples of in vitro polypeptide synthesis include, but are not limited to, the Rapid Translation System (RTS) manufactured by Roche Diagnostics Corp.

Examples of the host prokaryotic cells include *Escherichia coli* and *Bacillus subtilis*. To transform these host cells with the gene of interest, the host cells are transformed with plasmid vectors comprising a replicon, i.e., a replication origin, and a regulatory sequence derived from a species compatible with the hosts. Moreover, it is preferred that the vectors should have a sequence that can impart phenotypic character (phenotype) selectivity to the transformed cells.

The host eukaryotic cells encompass cells of vertebrates, insects, yeast, and the like. For example, monkey COS cells (Gluzman, Y. Cell (1981) 23, p. 175-182,

ATCC CRL-1650), mouse fibroblasts NIH3T3 (ATCC No. CRL-1658), and dihydrofolate reductase-deficient strains (Urlaub, G. and Chasin, L.A., Proc. Natl. Acad. Sci. USA (1980) 77, p. 4126-4220) of Chinese hamster ovarian cells (CHO cells, ATCC CCL-61) are often used as the vertebrate cells, though the vertebrate cells are not limited thereto.

The transformants thus obtained can be cultured according to standard methods and are able within the culture to intracellularly or extracellularly produce the polypeptide of interest.

A medium used in the culture can be selected appropriately according to the adopted host cells from among various media routinely used. For *Escherichia coli*, for example, an LB medium optionally supplemented with an antibiotic (e.g., ampicillin) or IPTG can be used.

The recombinant protein intracellularly or extracellularly produced by the transformants in the culture can be separated and purified by various separation procedures known in the art using the physical properties, chemical properties, or the like of the protein.

The procedures can be exemplified specifically by treatment with usual protein precipitants, ultrafiltration, various liquid chromatography techniques such as molecular sieve chromatography (gel filtration), adsorption chromatography, ion-exchange chromatography,

affinity chromatography, and high-performance liquid chromatography (HPLC), dialysis, and combinations thereof.

Moreover, the recombinant protein to be expressed can be linked to 6 histidine residues to thereby efficiently purify the resulting protein on a nickel affinity column.

By combining these methods, the polypeptide of interest can be produced easily in large amounts with high yields and high purity.

The antibody of the present invention can be obtained by immunizing animals with the antigen according to a standard method and collecting antibodies produced in vivo, followed by purification.

Moreover, antibody-producing cells that produce the antibody against EPHA2 are fused with myeloma cells according to a method known in the art (e.g., Kohler and Milstein, *Nature* (1975) 256, p. 495-497; and Kennet, R. ed., *Monoclonal Antibodies*, p. 365-367, Plenum Press, N.Y. (1980)) to thereby establish hybridomas, from which monoclonal antibodies can also be obtained.

(2) Production of anti-EPHA2 monoclonal antibody

Examples of antibodies which specifically bind to EPHA2 can include monoclonal antibodies which specifically bind to EPHA2. A method for obtaining the antibodies is as described below.

For monoclonal antibody production, the following process is generally required:

(a) the step of purifying biopolymers used as antigens,

(b) the step of immunizing animals with the antigens through injection, then collecting blood from the animals, assaying the antibody titer thereof to determine the timing of splenectomy, and then preparing antibody-producing cells,

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

(d) the step of performing cell fusion between the antibody-producing cells and the myelomas,

(e) the step of selecting a hybridoma group that produces the antibody of interest,

(f) the step of dividing into single cell clones (cloning),

(g) the step of culturing the hybridomas for producing monoclonal antibodies in large amounts or raising hybridoma-transplanted animals, according to circumstances,

(h) the step of studying the bioactivities and binding specificities of the monoclonal antibodies thus produced or assaying properties as labeling reagents, etc.

Hereinafter, the method for preparing monoclonal antibodies will be described in detail in line with these steps, though the method for preparing antibodies is not limited thereto. For example, antibody-producing cells other than splenic cells and myelomas can also be used.

(a) Purification of antigens

EPHA2 or partial polypeptides thereof prepared by the method as described above can be used as antigens.

Moreover, partial peptides of the protein of the present invention, which are chemically synthesized using membrane fractions prepared from EPHA2-expressing recombinant somatic cells or the EPHA2-expressing recombinant somatic cells themselves according to a method well known by those skilled in the art, can also be used as antigens.

(b) Preparation of antibody-producing cells

The antigens obtained in step (a) are mixed with adjuvants well known by those skilled in the art, for example, complete or incomplete Freund's adjuvants, or other auxiliaries such as potassium aluminum sulfate, and experimental animals are immunized with these immunogens. Animals used in hybridoma preparation methods known in the art can be used as the experimental animals without problems. Specifically, for example, mice, rats, goats, sheep, cow, and horses can be used. However, mice or rats are preferably used as the animals to be immunized, from the viewpoint of the easy availability of myeloma cells to be fused with the extracted antibody-producing cells, etc.

Moreover, the lineages of the mice and rats actually used are not particularly limited. For example, mouse lineages such as A, AKR, BALB/c, BDP, BA, CE, C3H, 57BL, C57BR, C57L, DBA, FL, HTH, HT1, LP, NZB, NZW, RF, R III,

SJL, SWR, WB, and 129 and rat lineages such as Low, Lewis, Sprague, Daweley, ACI, BN, and Fischer can be used.

These mice and rats can be obtained from, for example, experimental animal growers/distributors such as CLEA Japan, Inc. and Charles River Laboratories Japan, Inc.

Of these lineages, the mouse BALB/c lineage and the rat Low lineage are particularly preferable as the animals to be immunized, in consideration of fusion compatibility with myeloma cells as described later.

Moreover, mice having a reduced biological mechanism for autoantibody removal, i.e., autoimmune disease mice, are also preferably used in consideration of the antigenic homology between humans and mice.

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

For the immunization of the animals with EPHA2 or the recombinant antigens thereof, methods known in the art described in detail in, for example, Weir, D.M., Handbook of Experimental Immunology Vol. I. II. III., Blackwell Scientific Publications, Oxford (1987), and Kabat, E.A. and Mayer, M.M., Experimental Immunochemistry, Charles C Thomas Publisher Springfield, Illinois (1964) can be used.

Of these immunization methods, a method preferable in the present invention is specifically illustrated as described below.

Specifically, the membrane protein fractions used as antigens or antigen-expressing cells are first administered intradermally or intraperitoneally to the animals.

However, the combined use of both of the administration routes is preferable for enhancing immunization efficiency. Immunization efficiency can be enhanced particularly by performing intradermal administration in early immunizations and performing intraperitoneal administration in later immunizations or only in the last immunization.

The administration schedule of the antigens differs depending on the type of animals to be immunized, the individual differences thereof, etc. The antigens are generally administered at 3 to 6 doses preferably at 2- to 6-week intervals, more preferably at 3 to 4 doses at 2- to 4-week intervals.

Moreover, the dose of the antigens differs depending on the type of animals, the individual differences thereof, etc., and is generally of the order of 0.05 to 5 mg, preferably 0.1 to 0.5 mg.

A booster is performed 1 to 6 weeks later, preferably 2 to 4 weeks later, more preferably 2 to 3 weeks later, from such antigen administration.

In this context, the dose of the antigens in the booster differs depending on the type of animals, the size thereof, etc., and is generally of the order of 0.05

to 5 mg, preferably 0.1 to 0.5 mg, more preferably 0.1 to 0.2 mg, for example, for mice.

1 to 10 days later, preferably 2 to 5 days later, more preferably 2 to 3 days later, from the booster, splenic cells or lymphocytes containing antibody-producing cells are aseptically extracted from the animals to be immunized.

In this procedure, their antibody titers are measured, and animals having a sufficiently increased antibody titer can be used as sources of antibody-producing cells to thereby enhance the efficiency of the subsequent procedures.

Examples of methods for measuring the antibody titers used here can include, but not limited to, RIA and ELISA.

The antibody titer measurement according to the present invention can be performed by procedures as described below, for example, according to ELISA.

First, the purified or partially purified antigens are adsorbed onto the surface of a solid phase such as 96-well plates for ELISA. Furthermore, antigen-unadsorbed solid phase surface is covered with proteins unrelated to the antigens, for example, bovine serum albumin (hereinafter, referred to as "BSA"). The surfaces are washed and then contacted with serially diluted samples (e.g., mouse serum) as primary antibodies such that the antibodies in the samples are bound to the antigens.

Furthermore, enzyme-labeled antibodies against the mouse antibodies are added thereto as secondary antibodies such that the secondary antibodies are bound to the mouse antibodies. After washing, substrates for the enzyme are added thereto, and, for example, the change in absorbance caused by color development based on substrate degradation is measured to thereby calculate antibody titers.

The antibody-producing cells can be separated from these spleen cells or lymphocytes according to methods known in the art (e.g., Kohler et al., *Nature* (1975) 256, p. 495; Kohler et al., *Eur. J. Immunol.* (1977) 6, p. 511; Milstein et al., *Nature* (1977), 266, p. 550; and Walsh, *Nature*, (1977) 266, p. 495).

For example, for the spleen cells, a general method can be adopted, which involves cutting the cells into strips, filtering them through a stainless mesh, and then separating the antibody-producing cells therefrom by floating in Eagle's minimal essential medium (MEM).

(C) Preparation of myeloma cells (hereinafter, referred to as "myelomas")

Myeloma cells used in cell fusion are not particularly limited and can be selected appropriately, for use, from cell strains known in the art. However, HGPRT (hypoxanthine-guanine phosphoribosyl transferase)-deficient strains for which selection methods have been established are preferably used in consideration of convenient hybridoma selection from fused cells.

Specific examples thereof include: mouse-derived X63-Ag8 (X63), NS1-ANS/1 (NS1), P3X63-Ag8.U1 (P3U1), X63-Ag8.653 (X63.653), SP2/0-Ag14 (SP2/0), MPC11-45.6TG1.7 (45.6TG), FO, S149/5XXO, and BU.1; rat-derived 210.RSY3.Ag.1.2.3 (Y3); and human-derived U266AR (SKO-007), GM1500.GTG-A12 (GM1500), UC729-6, LICR-LOW-HMy2 (HMy2), and 8226AR/NIP4-1 (NP41).

These HGPRT-deficient strains can be obtained from, for example, American Type Culture Collection (ATCC).

These cell strains are subcultured in an appropriate medium, for example, an 8-azaguanine medium [RPMI-1640 medium supplemented with glutamine, 2-mercaptoethanol, gentamicin, and fetal bovine serum (hereinafter, referred to as "FBS") 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 in a normal medium [e.g., an ASF104 medium (manufactured by Ajinomoto Co., Inc.) containing 10% FBS] 3 to 4 days before cell fusion. On the day of fusion, 2×10^7 or more cells are secured.

(d) Cell fusion

Fusion between the antibody-producing cells and the myeloma cells can be performed appropriately under conditions that do not excessively reduce the cell survival rates, according to methods known in the art (e.g., Weir, D.M., Handbook of Experimental Immunology Vol. I. II. III., Blackwell Scientific Publications,

Oxford (1987); and Kabat, E.A. and Mayer, M.M., Experimental Immunochemistry, Charles C Thomas Publisher Springfield, Illinois (1964)).

For example, a chemical method which involves mixing the antibody-producing cells and the myeloma cells in a high-concentration polymer (e.g., polyethylene glycol) solution and a physical method which uses electric stimulations can be used as such methods.

Of these methods, the chemical method is specifically exemplified as described below.

Specifically, when polyethylene glycol is used as a polymer in the high-concentration polymer solution, the antibody-producing cells and the myeloma cells are mixed in a solution of polyethylene glycol having a molecular weight of 1500 to 6000, preferably 2000 to 4000, at a temperature of 30 to 40°C, preferably 35 to 38°C, for 1 to 10 minutes, preferably 5 to 8 minutes.

(e) Selection of hybridoma group

A method for selecting the hybridomas obtained by the cell fusion is not particularly limited, and a HAT (hypoxanthine-aminopterin-thymidine) selection method (Kohler et al., Nature (1975) 256, p. 495; and Milstein et al., Nature (1977) 266, p. 550) is usually used.

This method is effective for obtaining hybridomas using HGPRT-deficient myeloma cell strains that cannot survive in aminopterin.

Specifically, unfused cells and the hybridomas can be cultured in a HAT medium to thereby cause only aminopterin-resistant hybridomas to remain and grow.

(f) Dividing into single cell clones (cloning)

For example, methods known in the art, such as methylcellulose, soft agarose, and limiting dilution methods can be used as methods for cloning the hybridomas (see e.g., Barbara, B.M. and Stanley, M.S.: Selected Methods in Cellular Immunology, W.H. Freeman and Company, San Francisco (1980)). Of these methods, the limiting dilution method is particularly preferable.

In this method, feeders such as rat fetus-derived fibroblast strains or normal mouse splenic, thymus, or ascites cells are inoculated onto a microplate.

On the other hand, the hybridomas are diluted to 0.2 to 0.5 individuals/0.2 ml in advance in a medium. This solution containing the diluted hybridomas floating therein is added at a concentration of 0.1 ml/well, and the hybridomas can be continuously cultured for approximately 2 weeks while approximately 1/3 of the medium is replaced with a new one at regular intervals (e.g., 3-day intervals), to thereby grow hybridoma clones.

For wells having an observable antibody titer, for example, cloning by the limiting dilution method is repeated 2 to 4 times, and clones whose antibody titer is stably observed can be selected as anti-EPHA2 monoclonal antibody-producing hybridoma strains.

Examples of the hybridoma strains thus cloned can include hybridoma SH348-1 and hybridoma SH357-1.

Hybridoma SH348-1 and hybridoma SH357-1 have been deposited on Jun. 8, 2007 with International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology, (address: Tsukuba Central 6, Higashi 1-1-1, Tsukuba, Ibaraki, Japan). Hybridoma SH348-1 has been designated as SH348-1 with Accession No. FERM BP-10836, and hybridoma SH357-1 has been designated as SH357-1 with Accession No. FERM BP-10837.

In the present specification, an antibody produced by hybridoma SH348-1 is referred to as "SH348-1", and an antibody produced by hybridoma SH357-1 is referred to as "SH357-1".

(g) Preparation of monoclonal antibodies by hybridoma culture

The hybridomas thus selected can be cultured to thereby efficiently obtain monoclonal antibodies. Prior to the culture, it is preferred that hybridomas producing the monoclonal antibody of interest should be screened.

For this screening, methods known per se in the art can be adopted.

The antibody titer measurement according to the present invention can be performed, for example, by ELISA as described in paragraph (b).

The hybridomas obtained by the method as described above can be cryopreserved in liquid nitrogen or in a freezer at -80°C or lower.

Moreover, the completely cloned hybridomas can be passaged several times in a HT medium (HAT medium except for aminopterin) and then cultured in a normal medium changed therefrom.

Large-scale culture is performed by rotational culture using large culture bottles or spinner culture.

A supernatant obtained in this large-scale culture can be purified according to methods well known by those skilled in the art, such as gel filtration, to obtain monoclonal antibodies which specifically bind to the protein of the present invention.

Moreover, the hybridomas can be intraperitoneally injected to mice of the same lineage thereas (e.g., BALB/c) or Nu/Nu mice and grown to obtain ascites containing the monoclonal antibody of the present invention in large amounts.

For the intraperitoneal administration, mineral oil such as 2,6,10,14-tetramethyl pentadecane (pristane) is administered beforehand (3 to 7 days before the administration) to obtain ascites in larger amounts.

For example, an immunosuppressive agent is intraperitoneally injected in advance to the mice of the same lineage as the hybridomas to inactivate the T cells. 20 days later, 10^6 to 10^7 hybridoma clone cells are allowed to float (0.5 ml) in a serum-free medium, and

this solution is intraperitoneally administered to the mice. Ascites are usually collected from the mice when abdominal distention occurs by accumulated ascites.

By this method, monoclonal antibodies are obtained with a concentration approximately 100 times higher than that in the culture solution.

The monoclonal antibodies obtained by the method can be purified by methods described in, for example, Weir, D.M.: Handbook of Experimental Immunology, Vol. I, II, III, Blackwell Scientific Publications, Oxford (1978).

Specific examples thereof include ammonium sulfate precipitation, gel filtration, ion-exchange chromatography, and affinity chromatography.

For the purification, commercially available monoclonal antibody purification kits (e.g., MAbTrap GII Kit; manufactured by Pharmacia Inc.) and the like can also be used as convenient methods.

The monoclonal antibodies thus obtained have high antigen specificity for EPHA2.

(h) Assay of monoclonal antibodies

The isotype and subclass of the monoclonal antibodies thus obtained can be determined as described below.

First, examples of identification methods include the Ouchterlony method, ELISA, and RIA.

The Ouchterlony method is convenient but requires a concentration procedure for a low concentration of monoclonal antibodies.

On the other hand, when the ELISA or RIA is used, the culture supernatant is directly reacted with an antigen-adsorbed solid phase, and further, antibodies compatible with various immunoglobulin isotypes and subclasses can be used as secondary antibodies to thereby identify the isotype or subclass of the monoclonal antibodies.

Moreover, commercially available kits for identification (e.g., Mouse Typer Kit; manufactured by Bio-Rad Laboratories, Inc.) and the like can also be used as more convenient methods.

Furthermore, the proteins can be quantified according to a Folin-Lowry method and a calculation method using absorbance at 280 nm [$1.4 \text{ (OD}_{280}) = 1 \text{ mg/ml immunoglobulin}$].

(3) Other antibodies

The antibody of the present invention encompasses the monoclonal antibody against EPHA2 as well as genetic recombinant antibodies artificially modified for the purpose of, for example, reducing xenoantigenicity against humans, for example, chimeric, humanized, and human antibodies. These antibodies can be produced according to known methods.

Examples of the chimeric antibody include an antibody having variable and constant regions derived from species different from each other and specifically include a chimeric antibody comprising mouse-derived variable regions and human-derived constant regions

joined together (see Proc. Natl. Acad. Sci. U.S.A., 81, 6851-6855, (1984)).

Examples of the humanized antibody can include an antibody comprising a human-derived antibody with complementarity determining regions (CDRs) replaced with those of another species (see Nature (1986) 321, p. 522-525) and an antibody comprising a human antibody with CDR sequences and some framework amino acid residues replaced with those of another species by CDR grafting (see WO 90/07861 and US6972323).

Further examples of the antibody of the present invention can include an anti-human antibody. The anti-EPHA2 human antibody means a human antibody having only the gene sequence of a human chromosome-derived antibody. The anti-EPHA2 human antibody can be obtained by methods using human antibody-producing mice having a human chromosome fragment containing genes of human antibody H and L chains (see e.g., Tomizuka, K. et al., Nature Genetics (1997) 16, p. 133-143; Kuroiwa, Y. et al., Nucl. 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., Matsuda, T. and Iijima, S. eds.), Kluwer Academic Publishers, 1999; and Tomizuka, K. et al., Proc. Natl. Acad. Sci. USA (2000) 97, p. 722-727).

For such transgenic animals, specifically, genetic recombinant animals in which loci of endogenous immunoglobulin heavy and light chains in non-human mammals are broken and loci of human immunoglobulin heavy

and light chains are introduced instead via yeast artificial chromosome (YAC) vectors or the like can be created by preparing knockout animals and transgenic animals and crossing these animals.

Moreover, eukaryotic cells are transformed with cDNA encoding each of such human antibody heavy and light chains, preferably vectors containing the cDNA, by gene recombination techniques, and transformed cells producing genetic recombinant human monoclonal antibodies can also be cultured to thereby obtain these antibodies from the culture supernatant.

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

Moreover, methods for obtaining phage-displayed human antibodies selected from human antibody libraries are also known (see e.g., 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; and Siriwardena, D. et al., *Ophthalmology* (2002) 109 (3), p. 427-431).

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

Likewise, another phage display method can also be used, which involves causing human antibody Fab (antigen-binding fragment) to be expressed on the surface of phage and selecting phages binding to antigens (WO 97/08320 and WO 01/05950).

Genes of the phages selected based on antigen binding can be analyzed to thereby determine DNA sequences encoding human antibody variable regions binding to the antigens.

When the DNA sequence of scFv or Fab binding to the antigens is clarified, CDR sequences are extracted therefrom, and expression vectors having the sequences can be prepared and introduced into appropriate hosts, followed by gene expression to obtain human antibodies (WO 92/01047, WO 92/20791, WO 93/06213, WO 93/11236, WO 93/19172, WO 95/01438, WO 95/15388, Annu. Rev. Immunol (1994) 12, p. 433-455, and Nature Biotechnology (2005) 23 (9), p. 1105-1116).

The antibody genes can be temporarily isolated and then introduced into appropriate hosts to prepare antibodies. In such a case, appropriate hosts and expression vectors can be combined for use.

When eukaryotic cells are used as hosts, animal cells, plant cells, and eukaryotic microorganisms can be used.

Examples of the animal cells can include (1) mammalian cells, for example, monkey COS cells (Gluzman, Y. Cell (1981) 23, p. 175-182, ATCC CRL-1650), mouse

fibroblasts NIH3T3 (ATCC No. CRL-1658), and dihydrofolate reductase-deficient strains (Urlaub, G. and Chasin, L.A. Proc. Natl. Acad. Sci. U.S.A. (1980) 77, p. 4126-4220) of Chinese hamster ovarian cells (CHO cells, ATCC CCL-61).

Moreover, these hosts can also be modified, for use, to express antibodies having a modified sugar chain structure and an enhanced ADCC activity (antibody-dependent cytotoxic activity) or CDC activity. Examples of such hosts can include CHO cells comprising genes incorporated therein which encode antibody molecules producing antibody compositions in which sugar chains having fucose-unbound N-acetylglucosamine at the reducing ends thereof occupy 20% or more of complex-type N-glycoside linked sugar chains binding to antibody Fc regions (see WO 02/31140).

When prokaryotic cells are used, examples thereof can include *Escherichia coli* and *Bacillus subtilis*.

The antibody gene of interest is introduced into these cells by transformation, and the transformed cells are cultured in vitro to obtain antibodies.

The isotype of the antibody of the present invention is not limited, and examples thereof include IgG (IgG1, IgG2, IgG3, or IgG4), IgM, IgA (IgA1 or IgA2), IgD, and IgE and can preferably include IgG and IgM.

Moreover, the antibody of the present invention may be an antibody fragment having the antigen-binding site of the antibody or a modified form thereof.

Examples of the antibody fragment include Fab, F(ab')₂, Fv, single-chain Fv (scFv) comprising heavy and light chain Fvs linked via an appropriate linker, a diabody, a linear antibody, and a multispecific antibody formed by antibody fragments.

Furthermore the antibody of the present invention may be a multispecific antibody having specificity for at least two different antigens.

Such a molecule usually binds two antigens (i.e., a bispecific antibody). The "multispecific antibody" according to the present invention encompasses antibodies having specificity for more (e.g., three) antigens.

The multispecific antibody used as the antibody of the present invention may be a full-length antibody or a fragment of such an antibody (e.g., a F(ab')₂ bispecific antibody). The bispecific antibody can be prepared by binding heavy and light chains (HL pairs) of two antibodies or can also be prepared by fusing hybridomas producing monoclonal antibodies different from each other to prepare bispecific antibody-producing fused cells (Millstein et al., Nature (1983) 305, p. 537-539).

The antibody of the present invention may be a single-chain antibody (also referred to as scFv). The single-chain antibody is obtained by linking antibody heavy and light chain V regions via a polypeptide linker (Pluckthun, The Pharmacology of Monoclonal Antibodies, 113 (Rosenberg and Moore ed., Springer Verlag, New York,

p. 269-315 (1994)); and Nature Biotechnology (2005), 23, p. 1126-1136).

Methods for preparing the single-chain antibody are well known in the art (see e.g., U.S. Patent Nos. 4,946,778, 5,260,203, 5,091,513, and 5,455,030). In this scFv, the heavy and light chain V regions are linked via a linker that does not form 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 and light chain V regions in the scFv may be derived from the same antibodies or may be derived from different antibodies.

For example, an arbitrary single-chain peptide of 12 to 19 residues is used as the peptide linker for linking the V regions.

DNA encoding the scFv is obtained by: amplifying, as templates, the full-length sequences or partial sequences (encoding the desired amino acid sequences) of DNA encoding the heavy chain or heavy chain V region of the antibody and DNA encoding the light chain or light chain V region thereof, by a PCR method using primer pairs designed for both ends thereof; and subsequently further amplifying DNA encoding the peptide linker portion in combination with a primer pair designed to respectively link both ends of the linker sequence to the heavy and light chain sequences.

Moreover, once the DNA encoding the scFv is prepared, expression vectors containing the DNA and hosts

transformed with the expression vectors can be obtained according to standard methods. Moreover, by use of the hosts, the scFv can be obtained according to standard methods.

For these antibody fragments, their genes are obtained and expressed in the same way as above, and the hosts can be allowed to produce the antibody fragments.

The antibody of the present invention may be a polyclonal antibody, which is a mixture of a plurality of anti-EPHA2 antibodies differing in amino acid sequences. One example of the polyclonal antibody can include a mixture of a plurality of antibodies differing in CDRs. A mixture of cells producing antibodies different from each other is cultured, and antibodies purified from the culture can be used as such polyclonal antibodies (see WO 2004/061104).

Antibodies obtained by binding the antibody of the present invention with various molecules such as polyethylene glycol (PEG) can also be used as the modified form of the antibody.

Furthermore, the antibody of the present invention may be a conjugate of these antibodies formed with other drugs (immunoconjugate). Examples of such an antibody can include conjugates obtained by binding these antibodies to radioactive materials or compounds having a pharmacological effect (Nature Biotechnology (2005) 23, p. 1137-1146).

The obtained antibodies can be purified until homogeneous. In the antibody separation and purification, any separation/purification method used for usual proteins can be used.

The antibodies can be separated and purified by appropriately selecting and combining, for example, using chromatography columns, filters, ultrafiltration, salting-out, dialysis, polyacrylamide gel electrophoresis for preparation, and isoelectric focusing (Strategies for Protein Purification and Characterization: A Laboratory Course Manual, Daniel R. Marshak et al. eds., Cold Spring Harbor Laboratory Press (1996); and Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)), though the separation/purification method is not limited thereto.

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

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

Examples of columns used in the affinity chromatography include protein A and protein G columns.

Examples of columns based on the protein A column include Hyper D, POROS, Sepharose F.F. (Pharmacia Inc.).

Moreover, the antibodies can also be purified through their affinity for antigens using an antigen-immobilized carrier.

3. Properties of antibody of the present invention

The anti-EPHA2 antibody of the present invention obtained by the method has the following properties:

(1) one antibody of the present invention has the following properties a) to e):

a) having no ability to phosphorylate EPHA2 tyrosine residues;

b) having an ADCC activity against EPHA2-expressing cells;

c) having a CDC activity against EPHA2-expressing cells;

d) having an antitumor activity in vivo; and

e) specifically binding to a polypeptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing.

Examples of the antibody having such properties can include any one antibody selected from the group consisting of the following 1) to 4):

1) SH348-1,

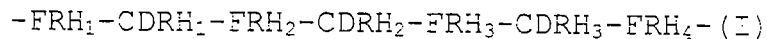
2) an antibody which recognizes an epitope recognized by an antibody produced by hybridoma SH348-1 (FERM BP-10836),

3) an antibody which has the amino acid sequences represented by SEQ ID NOs: 59, 61, and 63 in the sequence listing as complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOs: 65, 67, and 69 in

the sequence listing as complementarity determining regions in the light chain variable region,

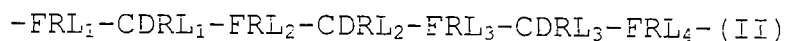
4) an antibody characterized by the following i) and ii):

i) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):



wherein FRH_1 represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids; $CDRH_1$ represents the amino acid sequence represented by SEQ ID NO: 59 in the sequence listing; FRH_2 represents an arbitrary amino acid sequence consisting of 14 amino acids; $CDRH_2$ represents the amino acid sequence represented by SEQ ID NO: 61 in the sequence listing; FRH_3 represents an arbitrary amino acid sequence consisting of 32 amino acids; $CDRH_3$ represents the amino acid sequence represented by SEQ ID NO: 63 in the sequence listing; and FRH_4 represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and

ii) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):



wherein FRL_1 represents an arbitrary amino acid sequence consisting of 23 amino acids; $CDRL_1$ represents the amino acid sequence represented by SEQ ID NO: 65 in the sequence listing; FRL_2 represents an arbitrary amino acid

sequence consisting of 15 amino acids; CDRL₂ represents the amino acid sequence represented by SEQ ID NO: 67 in the sequence listing; FRL₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRL₃ represents the amino acid sequence represented by SEQ ID NO: 69 in the sequence listing; and FRL₄ represents an arbitrary amino acid sequence consisting of 10 amino acids, wherein these amino acids are linked to each other through peptide bonds.

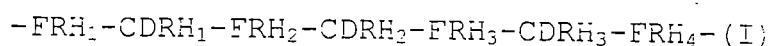
5) SH357-1,

6) an antibody which recognizes an epitope recognized by an antibody produced by hybridoma SH357-1 (FERM BP-10837),

7) an antibody which has the amino acid sequences represented by SEQ ID NOs: 71, 73, and 75 in the sequence listing as complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOs: 77, 79, and 81 in the sequence listing as complementarity determining regions in the light chain variable region,

8) an antibody according to any one of (5) to (7), characterized by the following i) and ii):

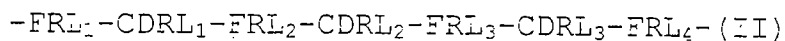
i) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):



wherein FRH₁ represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids sequences; CDRH₁ represents the amino acid sequence represented by SEQ ID

NO: 71 in the sequence listing; FRH₂ represents an arbitrary amino acid sequence consisting of 14 amino acids; CDRH₂ represents the amino acid sequence represented by SEQ ID NO: 73 in the sequence listing; FRH₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRH₃ represents the amino acid sequence represented by SEQ ID NO: 75 in the sequence listing; and FRH₄ represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and

ii) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):



wherein FRL₁ represents an arbitrary amino acid sequence consisting of 23 amino acids; CDRL₁ represents the amino acid sequence represented by SEQ ID NO: 77 in the sequence listing; FRL₂ represents an arbitrary amino acid sequence consisting of 15 amino acids; CDRL₂ represents the amino acid sequence represented by SEQ ID NO: 79 in the sequence listing; FRL₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRL₃ represents the amino acid sequence represented by SEQ ID NO: 81 in the sequence listing; and FRL₄ represents an arbitrary amino acid sequence consisting of 10 amino acids, wherein these amino acids are linked to each other through peptide bonds.

(2) another antibody of the present invention has the following properties a) to f):

a) having no ability to phosphorylate EPHA2 tyrosine residues;

b) exhibiting an effect of decreasing an EPHA2 protein level;

c) having an ADCC activity against EPHA2-expressing cells;

d) having a CDC activity against EPHA2-expressing cells;

e) having an antitumor activity in vivo; and

f) specifically binding to a polypeptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing.

Examples of the antibody having such properties can include any one antibody selected from the group consisting of the following 1) to 4):

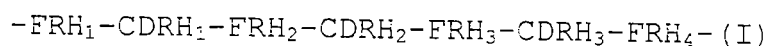
1) SH348-1,

2) an antibody which recognizes an epitope recognized by an antibody produced by hybridoma SH348-1 (FERM BP-10836),

3) an antibody which has the amino acid sequences represented by SEQ ID NOs: 59, 61, and 63 in the sequence listing as complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOs: 65, 67, and 69 in the sequence listing as complementarity determining regions in the light chain variable region,

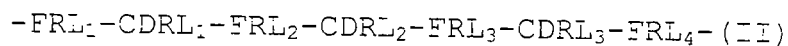
4) an antibody characterized by the following i) and ii):

i) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):



wherein FRH₁ represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids; CDRH₁ represents the amino acid sequence represented by SEQ ID NO: 59 in the sequence listing; FRH₂ represents an arbitrary amino acid sequence consisting of 14 amino acids; CDRH₂ represents the amino acid sequence represented by SEQ ID NO: 61 in the sequence listing; FRH₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRH₃ represents the amino acid sequence represented by SEQ ID NO: 63 in the sequence listing; and FRH₄ represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and

ii) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):



wherein FRL₁ represents an arbitrary amino acid sequence consisting of 23 amino acids; CDRL₁ represents the amino acid sequence represented by SEQ ID NO: 65 in the sequence listing; FRL₂ represents an arbitrary amino acid sequence consisting of 15 amino acids; CDRL₂ represents the amino acid sequence represented by SEQ ID NO: 67 in

the sequence listing; FRL₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDR_{L3} represents the amino acid sequence represented by SEQ ID NO: 69 in the sequence listing; and FRL₄ represents an arbitrary amino acid sequence consisting of 10 amino acids, wherein these amino acids are linked to each other through peptide bonds.

(3) another antibody of the present invention has the following properties a) to e):

a) having no ability to phosphorylate EP_{HA}2 tyrosine residues;

b) exhibiting no effect of decreasing an EP_{HA}2 protein level;

c) having an ADCC activity;

d) having a CDC activity;

e) having an antitumor activity in vivo; and

f) specifically binding to a polypeptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing.

Examples of the antibody having such properties can include any one antibody selected from the group consisting of the following 1) to 4):

1) SH357-1,

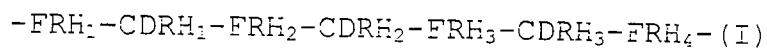
2) an antibody which recognizes an epitope recognized by an antibody produced by hybridoma SH357-1 (FERM BP-10836),

3) an antibody which has the amino acid sequences represented by SEQ ID NOs: 71, 73, and 75 in the sequence

listing as complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOs: 77, 79, and 81 in the sequence listing as complementarity determining regions in the light chain variable region,

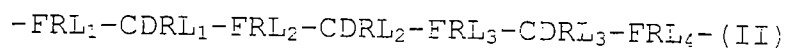
4) an antibody having the following properties i) and ii):

i) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):



wherein FRH_1 represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids; $CDRH_1$ represents the amino acid sequence represented by SEQ ID NO: 71 in the sequence listing; FRH_2 represents an arbitrary amino acid sequence consisting of 14 amino acids; $CDRH_2$ represents the amino acid sequence represented by SEQ ID NO: 73 in the sequence listing; FRH_3 represents an arbitrary amino acid sequence consisting of 32 amino acids; $CDRH_3$ represents the amino acid sequence represented by SEQ ID NO: 75 in the sequence listing; and FRH_4 represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and

ii) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):



wherein FRL₁ represents an arbitrary amino acid sequence consisting of 23 amino acids; CDRL₁ represents the amino acid sequence represented by SEQ ID NO: 77 in the sequence listing; FRL₂ represents an arbitrary amino acid sequence consisting of 15 amino acids; CDRL₂ represents the amino acid sequence represented by SEQ ID NO: 79 in the sequence listing; FRL₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRL₃ represents the amino acid sequence represented by SEQ ID NO: 81 in the sequence listing; and FRL₄ represents an arbitrary amino acid sequence consisting of 10 amino acids, wherein these amino acids are linked to each other through peptide bonds.

4. Pharmaceutical agent comprising anti-EPHA2 antibody

The anti-EPHA2 antibody of the present invention is useful as a pharmaceutical agent, particularly, a pharmaceutical composition intended for cancer treatment, or as an antibody for immunological diagnosis of such disease.

Preferable examples of cancer types can include, but not limited to, breast cancer, esophagus cancer, prostate cancer, gastric cancer, non-small cell lung cancer, colon cancer, and glioblastoma multiforme.

The present invention also provides a pharmaceutical composition comprising a therapeutically effective amount of the anti-EPHA2 antibody and a pharmaceutically

acceptable diluent, carrier, solubilizing agent, emulsifying agent, preservative, and/or adjuvant.

It is preferred that the substances pharmaceutically used that are acceptable in the pharmaceutical composition of the present invention should be nontoxic, at the dose or administration concentration used, to individuals that receive the pharmaceutical composition.

The pharmaceutical composition of the present invention can contain a pharmaceutical substance for changing, maintaining, or retaining pH, osmotic pressure, viscosity, transparency, color, isotonicity, sterility, stability, the rate of dissolution, the rate of sustained release, absorptivity, or permeability.

Examples of the pharmaceutical substance can include, but not limited to, the following: amino acids such as glycine, alanine, glutamine, asparagine, arginine, and lysine; antimicrobial agents; antioxidants such as ascorbic acid, sodium sulfate, and sodium bisulfite; buffers such as phosphate, citrate, and borate buffers, hydrogen carbonate, and Tris-HCl solutions; fillers such as mannitol and glycine; chelating agents such as ethylenediaminetetraacetic acid (EDTA); complexing agents such as caffeine, polyvinyl pyrrolidone, β -cyclodextrin, and hydroxypropyl- β -cyclodextrin; extenders such as glucose, mannose, and dextrin; monosaccharides, disaccharides, glucose, mannose, and other hydrocarbons such as dextrin; coloring agents; flavoring agents; diluents; emulsifying agents; hydrophilic polymers such

as polyvinyl pyrrolidine; 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; delivery vehicles; diluents; excipients; and/or pharmaceutical adjuvants.

The amounts of these pharmaceutical substances added are preferably 0.01 to 100 times, particularly, 0.1 to 10 times higher than the weight of the anti-EPHA2 antibody.

In this context, the present invention also encompasses a pharmaceutical composition containing an immunoliposome containing the anti-EPHA2 antibody in a liposome or the anti-EPHA2 antibody bound with a liposome (U.S. Patent No. 6214388).

The preferable composition of the pharmaceutical composition in a preparation can be determined appropriately by those skilled in the art according to applicable disease, an applicable administration route, etc.

The excipients or carriers in the pharmaceutical composition may be liquid or solid. The appropriate excipients or carriers may be injectable water, saline, cerebrospinal fluids, or other substances usually used in parenteral administration.

Neutral saline or serum albumin-containing saline can also be used as a carrier. The pharmaceutical composition can also contain a Tris buffer (pH 7.0 to 8.5) or an acetate buffer (pH 4.0 to 5.5) as well as sorbitol or other compounds. The pharmaceutical composition of the present invention is prepared in a freeze-dried or liquid form as an appropriate drug having the selected composition and necessary purity.

The pharmaceutical composition comprising the anti-EPHA2 antibody can also be prepared in a freeze-dried form using an appropriate excipient such as sucrose.

The pharmaceutical composition of the present invention can be prepared for parenteral administration or can also be prepared for gastrointestinal absorption.

The composition and concentration of the preparation can be determined depending on an administration method. When the anti-EPHA2 antibody contained in the pharmaceutical composition of the present invention has higher affinity for EPHA2, i.e., higher affinity (lower K_d value) for EPHA2 with respect to a dissociation constant (K_d value), the drug containing this antibody can be efficacious at a lower dose in humans. Based on

this result, the dose of the pharmaceutical composition of the present invention in human can also be determined.

The dose of the anti-EPHA2 antibody in humans may be usually be approximately 0.1 to 100 mg/kg once per 1 to 180 days.

Examples of dosage forms of the pharmaceutical composition of the present invention include injections including drip, suppositories, nasal agents, sublingual agents, and transdermally absorbable agents.

The administration of the pharmaceutical composition of the present invention can inhibit the growth of EPHA2-expressing tumors.

Examples

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

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

(Example 1) Preparation of plasmid

1)-1 Preparation of vectors expressing human EPHA2

1)-1-1 Preparation of a vector expressing full-length human EPHA2

cDNA encoding human EPHA2 was amplified by PCR reaction using cDNA synthesized from SK-OV-3 cell-derived total RNA as the template and a primer set:

Primer 1: 5'-

ggggacaagtttgtacaaaaagcaggcttcggggatcggaccgagagcgagaag-3' (sequence listing sequence ID No. 3); and

primer 2: 5'-

ggggaccactttgtacaagaaagctgggtcctagatggggatccccacagtgttcacctggtcctt-3'

(sequence listing sequence ID No. 4).

The PCR product was incorporated into pDONR221 (manufactured by Invitrogen Corp.) using BP Clonase (manufactured by Invitrogen Corp.) to prepare an entry vector. The stop codon was removed from the EPHA2 gene in the entry vector using GeneTailor Site-Directed Mutagenesis System (manufactured by Invitrogen Corp.) and a primer set:

Primer 3: 5'-ctgtggggatccccatcgaccagctttc-3'

(sequence listing sequence ID No. 5); and

Primer 4: 5'-gatggggatccccacagtgttcacctggtc-3'

(sequence listing sequence ID No. 6).

Recombination reaction between the obtained entry vector and the pcDNA-DEST40 Gateway Vector (manufactured by Invitrogen Corp.) was performed using LR Clonase

(manufactured by Invitrogen Corp.) to prepare pcDNA-DEST40-EPHA2 (the present vector has the nucleotide sequence represented by SEQ ID NO: 7 in the sequence listing, between attB1 and attB2 sequences). Moreover, the sequence of the ORF portion of the EPHA2 gene cloned into the present vector is represented by nucleotide Nos. 33 to 2960 of SEQ ID NO: 7 in the sequence listing. Moreover, the amino acid sequence of EPHA2 is represented by SEQ ID NO: 8 in the sequence listing.

1)-1-2 Preparation of EPHA2 extracellular region expression vector

cDNA encoding a human EPHA2 extracellular region polypeptide (consisting of an amino acid sequence represented by amino acid Nos. 1 to 534 of SEQ ID NO: 8 in the sequence listing; hereinafter, abbreviated to "EPHA2-ECD") was amplified by PCR reaction using a primer set:

Primer 5: 5'-aaaaagcttatggagctccaggcagcccg-3'
(sequence listing sequence ID No. 9); and

Primer 6: 5'-aaagggccctcagttgccagatccctccgg-3'
(sequence listing sequence ID No. 10).

The obtained PCR product was cleaved with HindIII and ApaI and cloned into the HindIII/ApaI site of pcDNA3.1 (hereinafter, the resulting vector is abbreviated to "pcDNA3.1-EPHA2-ECD"; and in the description below and the drawings, the recombinant protein expressed by "pcDNA3.1-EPHA2-ECD" is referred to as "rEPHA2-ECD").

1)-1-3 Preparation of expression vectors of truncated EPHA2 proteins

To construct vectors expressing a region consisting of an amino acid sequence represented by amino acid Nos. 315 to 540 of SEQ ID NO: 8 in the sequence listing of EPHA2 (hereinafter, referred to as "FnIII-NC"), a region consisting of an amino acid sequence represented by amino acid Nos. 315 to 430 thereof (hereinafter, referred to as "FnIII-N"), or a region consisting of an amino acid sequence represented by amino acid Nos. 426 to 540 thereof (hereinafter, referred to as "FnIII-C"), PCR reactions with pcDNA-DEST40-EPHA2 as the templates were performed using each primer set:

Primer set for FnIII-NC amplification:

Primer 7: 5'-

gcaggcttcacgcgaaggctcgtggcgccgcacctcaggacccag-3' (sequence listing sequence ID No. 11); and

Primer 8: 5'-

gtacaagaaagctgggtgctagccgccaatcaccgcccaag-3' (sequence listing sequence ID No. 12);

Primer set for FnIII-N amplification:

Primer 7, and

Primer 9: 5'-

gtacaagaaagctgggtgctagccagctacggaagctgcgg-3' (sequence listing sequence ID No. 13);

Primer set for FnIII-C amplification:

Primer 10: 5'-

gcaggcttcacatcgaaggctcgtgggagcttccgtactgccagtg-3' (sequence listing sequence ID No. 14); and

Primer 8.

To add attB1 and attB2 sites to both ends of the obtained PCR products, PCR reaction with each PCR product as a template was performed using a primer set:

Primer 11: 5'-

ggggacaagtttgtacaaaaaagcaggcttcacatcgaaggctcgtggg-3' (sequence listing sequence ID No. 15); and

Primer 12: 5'-ggggaccactttgtacaagaaagctgggt-3'

(sequence listing sequence ID No. 16).

The PCR products obtained in this procedure were incorporated into pDONR221 using BP Clonase to prepare entry vectors. Recombination reactions between each entry vector and a destination vector prepared by cleaving the NdeI and BamHI sites of pET15b (manufactured by Novagen) with restriction enzymes, blunting the cleaved sites, and then ligating Reading Frame Cassette C.1 of Gateway Vector Conversion System (manufactured by Invitrogen Corp.) into the blunted sites were performed using LR Clonase in order to prepare expression vectors (hereinafter, recombinant proteins expressed by the FnIII-NC-, FnIII-N-, and FnIII-C-incorporated expression vectors are referred to as rFnIII-NC, rFnIII-N, and rFnIII-C, respectively).

1)-2 Preparation of human EPHB2 extracellular region expression vector

cDNA encoding human EPHB2 was obtained by PCR reaction using cDNA synthesized from HCC70 cell-derived total RNA as the template and a primer set:

Primer 13: 5'-

ggggacaagtttgtagaaaaaagcaggcttcgccccgggaagcgcagcc-3'

(sequence listing sequence ID No. 17); and

Primer 14:

5'-

ggggaccactttgtacaagaaagctgggtcctaaacctccacagactgaatctggttc
catctg-3' (sequence listing sequence ID No. 18).

The nucleotide sequence of human EPHB2 cDNA is represented by SEQ ID NO: 19 in the sequence listing.

The amino acid sequence thereof is represented by SEQ ID NO: 20 in the sequence listing. PCR reaction was performed using a primer set for amplifying cDNA encoding a human EPHB2 extracellular region (region consisting of an amino acid sequence represented by amino acid Nos. 1 to 542 of SEQ ID NO: 20 in the sequence listing) (hereinafter, abbreviated to "EPHB2-ECD"):

Primer 15: 5'-aaaaagcttatggctctgcggaggctgggg-3'

(sequence listing sequence ID No. 21); and

Primer 16: 5'-aaagatatctcatggcaacttctcctggat-3'

(sequence listing sequence ID No. 22).

The obtained PCR product was cleaved with HindIII and EcoRV and cloned into the HindIII/EcoRV site of pcDNA3.1 (hereinafter, the resulting vector is abbreviated to "pcDNA3.1-EPHB2-ECD"; and in the description below and

the drawings, the recombinant protein expressed by "pcDNA3.1-EPHB2-ECD" is referred to as rEPHB2-ECD).

1)-3 Preparation of human ERBB2 expression vector

PCR reaction with Human clone collection (manufactured by STRATAGENE, #C33830) as the template was performed using a primer set:

Primer 17: 5'-caccatggagctggcgcccttg-3' (sequence listing sequence ID No. 23); and

Primer 18: 5'-tcccactggcacgtccagacc-3' (sequence listing sequence ID No. 24).

The obtained PCR product was incorporated into pENTR/D-TOPO (manufactured by Invitrogen Corp.) using pENTR Directional TOPO Cloning kit (manufactured by Invitrogen Corp.) to prepare an entry vector. To repair mutations caused by amino acid substitution, the entry vector was digested with EcoRI, and a fragment containing the pENTR/D-TOPO-derived sequence among the obtained fragments was ligated with the second largest fragment (approximately 1.6 kbp) among fragments obtained by digested Human clone collection (manufactured by STRATAGENE, #C14640) with EcoRI. Recombination reactions between the obtained entry vector and the pcDNA-DEST40 Gateway vector were performed using LR Clonase in order to prepare pcDNA-DEST40-ERBB2 (the vector has the nucleotide sequence represented by SEQ ID NO: 25 in the sequence listing, between attB1 and attB2 sequences).

(Example 2) Preparation of monoclonal antibody

2)-1 Preparation of antigen

To express EPHA2-ECD, FreeStyle 293-F cells (manufactured by Invitrogen Corp.) were transfected with pcDNA3.1-EPHA2-ECD using 293fectin (manufactured by Invitrogen Corp.) and cultured at 37°C in 8% CO₂ for 5 days. After the culture, the culture supernatant was collected by centrifugation and used as a source for rEPHA2-ECD purification. The obtained culture supernatant was dialyzed against 20 mM Tris-HCl, pH 7.5, using a dialysis tube having a molecular cutoff of 15000, then filtered through a filter (0.45 µm, PES), and then applied to HiPrep 16/10 Q XL (manufactured by GE Healthcare Bio-Sciences Corp.) equilibrated with 20 mM Tris-HCl, pH 7.5. Elution was performed with a linear concentration gradient of NaCl (20 mM Tris-HCl, pH 7.5, 0-1 M NaCl). An aliquot of the eluted fractions was separated by SDS-polyacrylamide gel electrophoresis (hereinafter, abbreviated to "SDS-PAGE"). Then, the gel was stained with Coomassie Brilliant Blue (hereinafter, abbreviated to "CBB-stained") to confirm rEPHA2-ECD-containing fractions. Next, the rEPHA2-ECD-containing fractions were combined and applied to HiLoad 26/60 Superdex 200 pg (manufactured by GE Healthcare Bio-Sciences Corp.) equilibrated with PBS. After elution with PBS, an aliquot of the eluted fractions was separated by SDS-PAGE. Then, the gel was CBB-stained to confirm rEPHA2-ECD-containing fractions. The rEPHA2-ECD-containing fractions were combined and used as an antigen

for immunization and an antigen for epitope determination. The protein concentration was measured using BCA Protein Assay Reagent (manufactured by PIERCE).

2)-2 Immunization

4- to 6-week-old BALB/cAnNCr1Crlj mice (Charles River Laboratories Japan, Inc.) were used. On day 0, a mixture of 50 µg of rEPHA2-ECD and Adjuvant Complete Freund H37 Rv (manufactured by Wako Pure Chemical Industries, Ltd.) (1:1 in terms of volume ratio) was subcutaneously administered to the mouse dorsal region. Likewise, a mixture of 50 µg of rEPHA2-ECD and TiterMax Gold Adjuvant (manufactured by Sigma-Aldrich, Inc.) (1:1 in terms of volume ratio) was subcutaneously administered to the dorsal region of another individual. On days 22 and 36, a mixture of 50 µg of rEPHA2-ECD and Adjuvant Incomplete Freund (manufactured by Wako Pure Chemical Industries, Ltd.) (1:1 in terms of volume ratio) was subcutaneously administered to the dorsal region of each mouse. On day 53, 50 µg of rEPHA2-ECD was intraperitoneally administered to each mouse. On day 56, the mouse spleen was collected and used in hybridoma preparation.

2)-3 Hybridoma preparation

Cell fusion between the spleen cells and mouse myeloma P3X63Ag8U.1 cells was performed using PEG4000 (manufactured IBL (Immuno-Biological Laboratories, Co., Ltd.)) to prepare hybridomas. A culture supernatant of

the obtained hybridomas was used for screening anti-EPHA2 antibody-producing hybridomas.

2)-4 Antibody screening

2)-4-1 Preparation of cells expressing a gene encoding an antigen

293T cells were seeded at 5×10^4 cells/cm² onto a collagen type I-coated flask (manufactured by IWAKI) and cultured overnight at 37°C in 5% CO₂ in DMEM containing 10% FBS. On the next day, the 293T cells were transfected with pcDNA-DEST40-EPHA2 or pcDNA-DEST40-ERBB2 as a control using Lipofectamine 2000 (manufactured by Invitrogen Corp.) and further incubated overnight at 37°C in 5% CO₂. On the next day, the transfected 293T cells were treated with trypsin, then washed with DMEM containing 10% FBS, and then suspended in PBS containing 5% FBS. The obtained cell suspension was used in Cell-ELISA and flow cytometry analysis.

2)-4-2 Cell-ELISA

The cell suspension prepared in the paragraph 2)-4-1 was centrifuged, and the supernatant was removed. Then, the EPHA2-expressing 293T cells and the ERBB2-expressing 293T cells were separately suspended by the addition of the hybridoma culture supernatant and incubated at 4°C for 1 hour. The cells in the wells were washed twice with PBS containing 5% FBS. Then, the cells were suspended by the addition of Goat anti-Mouse IgG, Peroxidase Conjugated (manufactured by Millipore (Chemicon), #AP181P) diluted 500 times with PBS

containing 5% FBS, and incubated at 4°C for 1 hour. The cells in the wells were washed twice with PBS containing 5% FBS. Then, OPD Color Developing Solution (o-phenylenediamine dihydrochloride (manufactured by Wako Pure Chemical Industries, Ltd.) and H₂O₂ were dissolved at concentrations of 0.4 mg/ml and 0.6% (v/v), respectively, in an OPD solution (0.05 M trisodium citrate, 0.1 M disodium hydrogen phosphate dodecahydrate, pH 4.5)) was added at 100 µl/well. Color reaction was performed with stirring and terminated by the addition of 1 M HCl at 100 µl/well. The cells were precipitated by centrifugation, and the supernatant was then transferred to a new 96-well flat-bottom microplate. The absorbance at 490 nm was measured using a plate reader (ARVO, PerkinElmer). To select hybridomas producing antibodies which specifically bind to EPHA2 expressed on the surface of the cell membrane, hybridomas producing a culture supernatant exhibiting higher absorbance in the EPHA2-expressing 293T cells than in the ERBB2-expressing 293T cells (controls) were selected to be positive for production of anti-EPHA2 antibody.

2)-4-3 Flow cytometric analysis

To eliminate false positives in Cell-ELISA, antibodies produced by the hybridoma determined to be positive in Cell-ELISA were further examined for their binding specificities for EPHA2 by flow cytometry. The cell suspension prepared in paragraph 2)-4-1 was centrifuged, and the supernatant was removed. Then, the

EPHA2-expressing 293T cells and the ERBB2-expressing 293T cells were separately suspended by the addition of the hybridoma culture supernatant and incubated at 4°C for 1 hour. The cells in the wells were washed twice with PBS containing 5% FBS. Then, the cells were suspended by the addition of "Fluorescein-conjugated goat IgG fraction to mouse IgG" (Whole Molecule) (manufactured by ICN Pharmaceuticals, Inc., #55493) diluted 1000 times with PBS containing 5% FBS, and incubated at 4°C for 1 hour. The cells were washed twice with PBS containing 5% FBS and then resuspended in PBS containing 5% FBS and further containing 2 µg/ml 7-aminoactinomycin D (manufactured by Invitrogen Corp. (Molecular Probes)), followed by analysis using a flow cytometer (FC500, Beckman Coulter, Inc.). The data was analyzed using Flowjo (Tree Star, Inc.). 7-aminoactinomycin D-positive dead cells were excluded using a gate. Then, the FITC fluorescence intensity histograms of live cells were plotted. Hybridomas producing samples that provided stronger fluorescence intensity in the fluorescence intensity histogram of the EPHA2-expressing 293T cells than in the histogram of the ERBB2-expressing 293T cells as controls were obtained as anti-EPHA2 antibody-producing hybridomas.

2)-5 Separation of hybridoma into single clones

The anti-EPHA2 antibody-producing hybridomas were diluted with ClonaCell-HY Selection Medium D (manufactured by StemCell Technologies, #03804) and cultured, and the formed colonies were collected as

single clones. The collected clones were separately cultured and examined for their binding activities for EPHA2 using the culture supernatants in the same way as in paragraph 2)-4-3 to establish hybridomas producing anti-EPHA2 monoclonal antibodies (SH348-1, SH357-1, Ab57-1, Ab65-1, Ab96-1, Ab100-1, Ab105-1, Ab106-13, Ab136-1, Ab148-1, Ab151-4, Ab230-1, Ab373-1, and Ab382-1).

2)-6 Confirmation of binding activity of monoclonal antibody for cancer cell line

Whether or not the monoclonal antibodies obtained in paragraph 2)-5 bound to cancer cells highly expressing EPHA2 was studied by the flow cytometric method in the same way as in paragraph 2)-4-3. A human breast cancer cell line (MDA-MB-231), a human lung cancer cell line (A549), and a human prostate cancer cell line (PC-3) were used instead of the transfected 293T cells. As a result, all the established monoclonal antibodies were confirmed to bind to these cancer cell lines.

2)-7 Isotype determination of monoclonal antibody

The isotypes of the monoclonal antibodies were determined using Mouse monoclonal isotyping kit (manufactured by AbD Serotec). As a result, the isotypes were IgG1 (Ab57-1 and Ab230-1), IgG2a (SH348-1, SH357-1, Ab65-1, Ab96-1, Ab100-1, Ab136-1, Ab148-1, and Ab151-4), and IgG2b (Ab105-1, Ab106-13, Ab373-1, and Ab382-1).

2)-8 Preparation of monoclonal antibody

The monoclonal antibodies were purified from ascites of hybridoma-transplanted mice or a hybridoma culture

supernatant (hereinafter, referred to as a "source for antibody purification").

The mouse ascites were prepared as follows: first, 7- to 8-week-old BALB/cAJcl-nu/nu mice (CLEA Japan, Inc.) were treated with pristane (manufactured by Sigma-Aldrich, Inc.). Approximately 3 weeks later, the hybridomas washed with saline were intraperitoneally transplanted in an amount of 1×10^7 cells/mouse. 1 to 2 weeks later, ascites accumulated in the peritoneal cavity was collected, then sterilized through a 0.22- μ m filter, and used as a source for antibody purification.

The hybridoma culture supernatant was prepared using CELLline (manufactured by BD Biosciences). The hybridomas were cultured according to the manufacturer's instructions except that ClonaCell-HY Growth Medium E (manufactured by StemCell Technologies, #03805) was used as a medium. The collected culture supernatant was filtered through a 0.45- μ m filter and used as a source for antibody purification.

The antibodies were purified using an affinity column comprising Recombinant Protein A rPA50 (manufactured by RepliGen Corp.) immobilized on Formyl-Cellulofine (manufactured by Seikagaku Corp.) (hereinafter, abbreviated to "Formyl-Cellulofine Protein A") or HiTrap MabSelect SuRe (manufactured by GE Healthcare Bio-Sciences Corp.). For the Formyl-Cellulofine Protein A, the source for antibody purification was diluted three times with Binding Buffer

(3 M NaCl, 1.5 M glycine, pH 8.9) and applied to the column, which was then washed with Binding Buffer, followed by elution with 0.1 M citric acid, pH 4.0. On the other hand, for the HiTrap MabSelect SuRe, the source for antibody purification was added to the column, which was then washed with PBS, followed by elution with 2 M arginine-HCl, pH 4.0. The antibody eluate was neutralized, and the buffer was then replaced with PBS.

The antibody concentrations were determined by eluting the antibodies bound with POROS G 20 μ m Column, PEEK, 4.6 mm $\times$ 100 mm, 1.7 ml (Applied Biosystems) and measuring the absorbance (O.D. 280 nm) of the eluate. Specifically, the antibody sample diluted with PBS was applied to POROS G 20 μ m equilibrated with Equilibrating 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 Equilibrating Buffer, and the antibody bound to the column was then eluted with an eluent (0.1% (v/v) HCl, 0.15 M NaCl). The peak area of the absorbance (O.D. 280 nm) of the eluate was measured, and the concentration was calculated according to the following equation:

Concentration of antibody sample (mg/ml) = (Peak area of antibody sample)/(Peak area of standard (human IgG1)) $\times$ Concentration of standard (mg/ml) $\times$ Dilution factor.

Moreover, the concentration of endotoxin contained in the obtained antibodies was measured using Endospecy

ES-50M Set (Seikagaku Corp., #020150) and Endotoxin Standard CSE-L Set (Seikagaku Corp., #020055) and was confirmed to be 1 EU/mg or lower. The resulting antibodies were used in the subsequent experiments.

(Example 3) Properties of SH-348-1 and SH357-1

3)-1 Study of anti-EPHA2 antibody for its activity of inducing phosphorylation of EPHA2 tyrosine residues and its activity of inducing decrease in EPHA2 protein level

3)-1-1 Preparation of antibody-stimulated cell lysate MDA-MB-231 cells suspended in RPMI1640 containing 10% FBS, 50 units/ml penicillin, and 50 µg/ml streptomycin (hereinafter, abbreviated to "10% FBS-containing RPMI1640 (with antibiotics)") were seeded at 6×10^5 cells/well onto a 6-well dish and cultured overnight at 37°C in 5% CO₂. On the next day, the medium was discarded, and RPMI1640 was added thereto. The cells were further cultured overnight at 37°C in 5% CO₂. On the next day, SH348-1, SH357-1, Mouse IgG_{2A} Isotype Control (in the description below and the drawings, abbreviated to "mIgG_{2a}"; manufactured by R&D Systems, Inc., #MAB003) as an isotype control antibody, Recombinant Mouse Ephrin-A1/Fc Chimera (in the description below and the drawings, abbreviated to "Ephrin-A1/Fc"; manufactured by R&D Systems, Inc., #602-A1-200) as a soluble EPHA2 ligand, and Recombinant Human IgG₁ Fc (in the description below and the drawings, abbreviated to "hG₁Fc"; manufactured by

R&D Systems, Inc., #110-HG-100) as a control protein for the soluble ligand were separately diluted at a concentration shown in Figure 1 or 2 (SH348-1, SH357-1, and mIgG2a: 10 $\mu\text{g/ml}$ or 50 $\mu\text{g/ml}$ in Figure 1A and 50 $\mu\text{g/ml}$ in Figure 2A, Ephrin-A1/Fc and hG₁Fc: 1 $\mu\text{g/ml}$ in Figures 1 and 2) with RPMI1640. The resulting solution was added to the MDA-MB-231 cells after discarding of the medium and incubated at 37°C in 5% CO₂ for the predetermined time in 5% CO₂. Moreover, in the experiments in the presence of a cross-linking antibody, SH348-1, SH357-1, or mIgG2a and Goat anti-mouse IgG, Fc γ fragment specific (min X Hu, Bov, Hrs Sr Prot) (manufactured by Jackson ImmunoResearch Laboratories, Inc., #115-005-071) as a cross-linking antibody were mixed at each concentration of 10 $\mu\text{g/ml}$ or 50 $\mu\text{g/ml}$ (Figure 1B) and 50 $\mu\text{g/ml}$ (Figure 2B) in RPMI1640. The resulting solution was added to the MDA-MB-231 cells after discarding of the medium and incubated at 37°C in 5% CO₂ for the predetermined time. At the predetermined time, the supernatant was removed, and the cells were lysed by the addition of 1xCell Lysis Buffer (manufactured by Cell Signaling Technology, Inc.) containing 1 mM PMSF (manufactured by Sigma-Aldrich, Inc.) and centrifuged at 15000 rpm for 5 minutes. The supernatants of cell lysates were used as samples in immunoprecipitation and Western blotting. The protein concentrations of the samples were measured using BCA Protein Assay Reagent (manufactured by PIERCE).

3)-1-2 Verification of activity of inducing phosphorylation of EPHA2 tyrosine residues

To immunoprecipitate EPHA2, first, 8 µg of anti-Eck/EphA2, clone D7 (in the description below and the drawings, abbreviated to "anti-EPHA2 antibody (D7)"; manufactured by Millipore (Upstate), #05-480) was added to 25 µl of a suspension of Protein G magnetic beads (manufactured by NEW ENGLAND BioLabs, Inc.) per sample, and the mixture was inverted for mixing at 4°C for 2 hours.

Then, FBS was added thereto at a final concentration of 10%, and the mixture was further inverted for mixing at 4°C for 30 minutes. The beads were washed three times with 1xCell Lysis Buffer containing 1 mM PMSF. Then, 200 µg of the cell lysate supernatants prepared in paragraph 3)-1-1 were added thereto, and the mixtures were inverted for mixing overnight at 4°C. On the next day, the beads were washed three times with 1xCell Lysis Buffer containing 1 mM PMSF. Then, SDS-Sample Buffer (56.3 mM Tris-HCl, pH 6.8, 1.8% (w/v) SDS, 9% glycerol, 0.72 M 2-mercaptoethanol, 0.045 mg/ml bromophenol blue) was added to the beads, and the mixtures were heated at 98°C for 5 minutes. The proteins dissociated from the beads were separated by SDS-PAGE.

To perform Western blotting, the proteins were transferred from the gels to polyvinylidene difluoride membranes (hereinafter, abbreviated to a "PVDF membrane"; 0.45 µm in pore size; manufactured by Millipore). After

the transfer, the PVDF membranes were blocked by shaking in Blocking Solution (one pouch of Block Ace powder (manufactured by Dainippon Sumitomo Pharma Co., Ltd. (Snow Brand Milk Products Co., Ltd.)) was dissolved in 100 ml of ultrapure water, to which Tween 20 and sodium azide were then added at final concentrations of 0.1% (v/v) and 0.02% (w/v), respectively). First, to detect the immunoprecipitated EPFA2, the PVDF membranes thus blocked were soaked in an anti-EPFA2 antibody (D7) solution diluted to 0.25 µg/ml with Blocking Solution, and shaken at room temperature for 1 hour. The PVDF membranes were washed for 10 minutes three times with TBST (50 mM Tris-HCl, pH 8.0, 138 mM NaCl, 2.7 mM KCl, 0.1% (v/v) Tween 20). Then, the PVDF membranes were soaked in an Anti-Mouse Ig, HRP-Linked Whole Ab Sheep (manufactured by GE Healthcare Bio-Sciences Corp.) solution diluted 3000 times with TBST, and shaken at room temperature for 30 minutes. The PVDF membranes were further washed for 10 minutes three times with TBST. Then, signals were detected on a film for chemiluminescence using ECL Plus (manufactured by GE Healthcare Bio-Sciences Corp.).

Next, to remove the antibodies from these PVDF membranes, the PVDF membranes were soaked in Stripping Solution (50 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 100 mM 2-mercaptoethanol) and shaken at 55°C for 30 minutes. Then, the PVDF membranes were soaked in Quenching Solution (TBST containing 1% (v/v) H₂O₂ and 0.1% (w/v) NaN₃), then

shaken at room temperature for 20 minutes, and further washed for 10 minutes three times with TBST. To detect the phosphorylated state of the EPHA2 tyrosine residues, these PVDF membranes were blocked by shaking in sodium azide-free Blocking Solution (one pouch of Block Ace powder was dissolved in 100 ml of ultrapure water, to which Tween 20 was then added at a final concentration of 0.1% (v/v)). Then, the PVDF membranes were soaked in an Anti-Phosphotyrosine, recombinant 4G10 HRP-conjugate (in the figures, abbreviated to "4G10 antibody"; manufactured by Millipore (Upstate), #16-184) solution diluted 10000 times with sodium azide-free Blocking Solution, and shaken at room temperature for 1 hour. The PVDF membranes were washed for 10 minutes three times with TBST and then further washed for 5 minutes three times with H₂O. Signals were detected on a film for chemiluminescence using ECL Plus.

As a result, by the addition of the soluble ligand Ephrin-A1/Fc, EPHA2 tyrosine residues were phosphorylated in 10 minutes. By contrast, when the antibodies SH348-1 and SH357-1 were added at a concentration of 10 µg/ml or 50 µg/ml, the effect of inducing the phosphorylation of EPHA2 tyrosine residues as seen by the ligand was not observed at all in the predetermined times (10 minutes, 30 minutes, and 60 minutes) (Figure 1A). Likewise, even in the presence of the cross-linking antibody, the effect of inducing the phosphorylation of EPHA2 tyrosine

residues as seen in the ligand was not observed in the presence of antibodies SH348-1 and SH357-1 (Figure 1B).

3)-1-3 Verification of activity of inducing decrease in EPHA2 protein level

10 μ g of the cell lysate supernatants prepared in paragraph 3)-1-1 were separated by SDS-PAGE. Then, the proteins in the gel were transferred to PVDF membranes and subjected to Western blotting using an anti-EPHA2 antibody (D7) and Monoclonal Anti- β -Actin clone AC-15 (in the description below and the figures, abbreviated to "anti- β -actin antibody"; manufactured by Sigma-Aldrich, Inc., #A-5441) as a control for the sample protein level. Specifically, after the transfer, the PVDF membranes were blocked by shaking in Blocking Solution and then cut with a razor into two pieces centered around a molecular weight of 70 kDa. The PVDF membranes containing 70 kDa or larger proteins were soaked in an anti-EPHA2 antibody (D7) solution diluted to 0.25 μ g/ml with Blocking Solution, while the PVDF membranes containing 70 kDa or smaller proteins were soaked in an anti- β -actin antibody solution diluted 1000 times with Blocking Solution. Each PVDF membrane was shaken at room temperature for 1 hour. Each PVDF membrane was washed for 10 minutes three times with TBST. Then, each PVDF membrane was soaked in an Anti-Mouse Ig, HRP-Linked Whole Ab Sheep solution diluted 3000 times with TBST, and shaken at room temperature for 30 minutes. Each PVDF membrane was washed for 10 minutes three times with TBST. Then, signals were detected using

ECL Plus and NightOWL LB983 (Berthold Technologies GmbH & Co. KG). The signal intensity of the bands was quantified using Gel-Pro Analyzer Version 4.5 for Windows (registered trademark; Media Cybernetics, Inc.).

As a result, by the addition of the soluble ligand Ephrin-A1/Fc, a significant decrease in EPHA2 protein level was observed (Figures 2A and 2B). By the addition of the antibody SH348-1, a decrease in EPHA2 protein level, albeit weaker than the activity of the ligand, was observed both in the presence and absence of the cross-linking antibody (Figures 2A and 2B). On the other hand, in the antibody SH357-1, almost no change in EPHA2 protein level was observed regardless of the presence or absence of the cross-linking antibody (Figures 2A and 2B).

To analyze the EPHA2 protein level after 24 hours of the SH348-1 addition, an average of {the signal intensity of the EPHA2 band/the signal intensity of the β -actin band} was calculated from three experimental results corrected with a ligand/antibody-nonsupplemented sample. As a result, the value after 24 hours of the SH348-1 addition was 70% in the absence of the cross-linking antibody and 69% in the presence of the cross-linking antibody, when the value after 24 hours of the mIgG2a addition is defined as 100%.

3)-2 ADCC activity

3)-2-1 Preparation of effector cells

The spleen was aseptically collected from CAnN.Cg-Foxn1^{nu}/Cr1Cr1j nude mice (Charles River Laboratories

Japan, Inc.). The collected spleen was homogenized with two slide glasses and hemolyzed using BD Pharm Lyse (manufactured by BD Biosciences, #555899). The obtained spleen cells were suspended in phenol red-free RPMI1640 (manufactured by Invitrogen Corp.) containing 10% Fetal Bovine Serum, Ultra-low IgG (manufactured by Invitrogen Corp.) (hereinafter, abbreviated to a "medium for ADCC") and passed through a cell strainer (40 μ m in pore size; manufactured by BD Biosciences). Then, the number of live cells was counted by a trypan blue exclusion test. The spleen cell suspension was centrifuged, and the medium was then removed. The cells were resuspended at a live cell density of 1.5×10^7 cells/ml in a medium for ADCC and used as effector cells.

3)-2-2 Preparation of target cells

MDA-MB-231, A549, or PC-3 cells were treated with trypsin. The cells were washed with RPMI1640 containing 10% FBS and then resuspended in RPMI1640 containing 10% FBS. Each cell (4×10^6 cells) was mixed with Chromium-51 (5550 kBq) sterilized through a 0.22- μ m filter, and labeled therewith at 37°C in 5% CO₂ for 1 hour. The labeled cells were washed three times with a medium for ADCC and resuspended at a concentration of 2×10^5 cells/ml in a medium for ADCC to prepare target cells.

3)-2-3 ⁵¹Cr release assay

The target cells (2×10^5 cells/ml) were dispensed at 50 μ l/well to a 96-well U-bottom microplate. 50 μ l of SH348-1, SH357-1, or an isotype control antibody (mIgG2a)

diluted to 2.5 µg/ml (in terms of a final concentration after addition of the effector cells) with a medium for ADCC was added thereto and incubated at 4°C for 1 hour. 100 µl of the effector cells (1.5×10^7 cells/ml) was added thereto and incubated overnight at 37°C in 5% CO₂. On the next day, the supernatant was collected into LumaPlate (manufactured by PerkinElmer). The dose of released gamma rays was measured using a gamma counter. The cell lysis rate attributed to the ADCC activity was calculated according to the following equation:

$$\text{Cell lysis rate (\%)} = (A-B)/(C-B) \times 100$$

A: counts in sample wells

B: an average ($n = 3$) of counts in spontaneous release (antibody/effector cell-nonsupplemented wells). Instead of the antibody and the effector cells, 50 µl and 100 µl, respectively, of a medium for ADCC were added. The other procedures were performed in the same way as in the sample wells.

C: an average ($n = 3$) of counts in the maximum release (wells containing the target cells dissolved in a detergent). 50 µl of a medium for ADCC was added instead of the antibody, and 100 µl of a medium for ADCC containing 2% (v/v) Triton-X100 was added instead of the effector cells. The other procedures were performed in the same way as in the sample wells.

Figure 3 shows an average of three experiments, wherein the error bar represents a standard deviation, and the P value was calculated by Student's t-test. As a

result, the antibody SH348-1 exhibited cell lysis activities of 8.2%, 9.1%, and 4.7% against the MDA-MB-231 cells (Figure 3A), the A549 cells (Figure 3B), and the PC-3 cells (Figure 3C), respectively. The antibody SH357-1 exhibited cell lysis activities of 8.8%, 13.0%, and 9.0% against the MDA-MB-231 cells (Figure 3A), the A549 cells (Figure 3B), and the PC-3 cells (Figure 3C), respectively. These results demonstrated that both the antibodies have an ADCC activity against MDA-MB-231 cells, A549 cells, and PC-3 cells.

3)-3 CDC activity

MDA-MB-231, A549, or PC-3 cells suspended in 10% FBS-containing RPMI1640 (with antibiotics) were seeded at 5000 cells/well onto a 96-well microplate and cultured overnight at 37°C in 5% CO₂. On the next day, SH348-1, SH357-1, or an isotype control antibody (mIgG2a) diluted to 25 µg/ml (in terms of a final concentration after addition of complements) with 10% FBS-containing RPMI1640 (with antibiotics) was added thereto and incubated at 4°C for 1 hour. Rabbit complements (manufactured by CEDARLANE, #CL3051) diluted to 30% with RPMI1640 were added thereto at a final concentration of 5%, then incubated at 37°C in 5% CO₂ for 1 hour, and further left standing at room temperature for 30 minutes. To measure the cell viability, CellTiter-Glo Luminescent Cell Viability Assay (manufactured by Promega Corp.) was added in an amount equal to that of the culture solution, and the mixture was stirred at room temperature for 10

minutes. Then, the amount of light emitted was measured using a plate reader. The cell viability was calculated according to the following equation:

$$\text{Cell viability (\%)} = (a-b)/(c-b) \times 100$$

a: the amount of light emitted from sample wells

b: an average ($n = 8$) of the amount of light emitted as a background (cell/antibody-nonsupplemented wells). 10% FBS-containing RPMI1640 (with antibiotics) was added, instead of the seeded cells, in an amount equal to that of the cell suspension, and 10% FBS-containing RPMI1640 (with antibiotics) was added, instead of the antibody, in an amount equal to that of the antibody dilution. The other procedures were performed in the same way as in the sample wells.

c: an average ($n = 3$) of the amount of light emitted from antibody-nonsupplemented wells. 10% FBS-containing RPMI1640 (with antibiotics) was added, instead of the antibody, in an amount equal to that of the antibody dilution. The other procedures were performed in the same way as in the sample wells.

Figure 4 shows an average of three experiments, wherein the error bar represents a standard deviation, and the P value was calculated by Student's t-test. As a result, the antibody SH348-1 induced 44%, 31%, and 41% decreases in the cell viability of the MDA-MB-231 cells (Figure 4A), the A549 cells (Figure 4B), and the PC-3 cells (Figure 4C), respectively, in the presence of the complements. The antibody SH357-1 also induced 65%, 60%,

and 65% decreases in the cell survival rates of the MDA-MB-231 cells (Figure 4D), the A549 cells (Figure 4E), and the PC-3 cells (Figure 4F), respectively, in the presence of the complements. These results demonstrated that both the antibodies have a CDC activity against MDA-MB-231 cells, A549 cells, and PC-3 cells.

3)-4 Epitope determination

3)-4-1 Preparation of truncated EPHA2 polypeptides (rFnIII-NC, rFnIII-N, and rFnIII-C)

Escherichia coli BL21 and *Escherichia coli* Origami (DE3) (manufactured by Novagen) were separately transformed with the expression plasmid prepared in paragraph 1)-1-3, and cultured in an LB medium supplemented with 50 µg/ml ampicillin (manufactured by Sigma-Aldrich, Inc.). Expression of truncated EPHA2 polypeptides was induced using Autoinduction System (manufactured by Novagen) for BL21 and the addition of 0.5 mM IPTG for Origami (DE3). The bacterial cells were collected by centrifugation at 6000 rpm for 20 minutes, then suspended in a homogenizing buffer (50 mM Tris HCl, pH 7.5, 150 mM NaCl, 0.1% (v/v) Triton-X100, 10% (v/v) glycerol), and then sonicated on ice. The supernatant was collected by centrifugation at 14000 rpm for 15 minutes and applied to 0.5 ml of Ni-NTA (manufactured by Invitrogen Corp.). The Ni-NTA was washed with a washing buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 50 mM imidazole, 10% (v/v) glycerol), followed by elution with an eluting buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl,

400 mM imidazole, 10% (v/v) glycerol). The eluted samples were further purified by gel filtration column chromatography (Superdex 75 10/300; manufactured by GE Healthcare Bio-Sciences Corp.) using PBS as a solvent. The protein concentrations of the obtained recombinant proteins were measured using Protein Assay (manufactured by Bio-Rad Laboratories, Inc).

3)-4-2 Preparation of EPHB2 extracellular region polypeptide (rEPHB2-ECD)

To express EPHB2-ECD, FreeStyle 293-F cells were transfected with pcDNA3.1-EphA2-ECD using 293fectin and cultured at 37°C in 8% CO₂ for 72 hours. After the culture, the culture solution was collected by centrifugation and used as a source for rEPHB2-EDC purification. The obtained culture supernatant was dialyzed against 20 mM Tris-HCl, pH 7.5, using a dialysis tube having a molecular cutoff of 15000, then filtered through a filter (0.45 µm, PES), and then applied to HiPrep 16/10 Q XL equilibrated with 20 mM Tris-HCl, pH 7.5. Elution was performed with a linear concentration gradient of NaCl (20 mM Tris-HCl, pH 7.5, 0-1 M NaCl). An aliquot of the eluted fractions was separated by SDS-PAGE. Then, the gel was CBB-stained to confirm that there were rEPHB2-ECD-containing fractions. Next, the rEPHB2-ECD-containing fractions were combined and applied to HiLoad 26/60 Superdex 200 pg equilibrated with PBS. After elution with PBS, an aliquot of the elution fractions was separated by SDS-PAGE. Then, the gel was

CBB-stained to confirm that there were rEPHB2-ECD-containing fractions. The rEPHB2-ECD-containing fractions were combined and used as an antigen for epitope determination. The protein concentration was measured using BCA Protein Assay Reagent.

3)-4-3 Determination of binding sites in antigens by ELISA

rEPHA2-ECD, rFnIII-NC, rFnIII-N, rFnIII-C, or a control protein rEPHB2-ECD was diluted to 1 µg/ml with PBS, then dispensed at 100 µl/well onto an immunoplate (manufactured by Nunc, #442404), and incubated overnight at 4°C to thereby adsorb the protein to the plate. On the next day, the solution in the wells was removed, and a Block Ace solution (one pouch of Block Ace powder was dissolved in 100 ml of ultrapure water) diluted 4 times with PBS was dispensed at 200 µl/well and incubated at room temperature for 1 hour. The solution in the wells was removed, and SH348-1, SH357-1, or an isotype control antibody (mIgG2a) diluted to 5 µg/ml with Diluting Buffer (PBS, 0.05% (v/v) Tween 20) was then added at 50 µl/well. The plate was incubated at room temperature for 1 hour. Then, the solution in the wells was removed, and the wells were washed twice with a diluting buffer. Goat anti-Mouse IgG, Peroxidase Conjugated, diluted 3000 times with Diluting Buffer was added at 50 µl/well and incubated at room temperature for 1 hour. The solution in the wells was removed, and the wells were washed twice with Diluting Buffer. Then, a color reaction was

performed with stirring by the addition of OPD Color Developing Solution at 100 μ l/well. After color development, the color reaction was terminated by the addition of 1 M HCl at 100 μ l/well. The absorbance at 490 nm was measured using a plate reader.

Figure 5A shows the EPHA2 domain structure prediction (NCBI CDD version 2.11, CBS TMHMM Server v.2.0) and the positions of EPHA2-ECD, FnIII-NC, FnIII-N, and FnIII-C in EPHA2. Ligand-BD represents a ligand-binding domain, FN3 represents a fibronectin type 3 domain, TM represents a transmembrane region, Trk kinase represents a tyrosine kinase domain, and SAM represents a SAM domain.

Recombinant proteins of the EPHA2 extracellular region (EPHA2-ECD), the region containing two fibronectin type 3 domains (FnIII-NC), the region containing the N-terminal fibronectin type 3 domain (FnIII-N), and the region containing the C-terminal fibronectin type 3 domain (FnIII-C) were prepared and studied for their binding activities with respect to SH348-1 and SH357-1. As a result, the antibodies SH348-1 and SH357-1 exhibited binding activities with respect to rEPHA2-ECD, rFnIII-NC, and rFnIII-C (Figure 5B). Thus, the antibodies SH348-1 and SH357-1 were shown to bind to a region from amino acids 426 to 534 containing the C-terminal fibronectin type 3 domain (amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing).

(Example 4) In vivo antitumor effect

MDA-MB-231 cells were dissociated from a culture flask by trypsin treatment and then suspended in 10% FBS-containing RPMI1640 (with antibiotics). After centrifugation, the supernatant was removed. The cells were washed twice with the same medium as above, then suspended in BD Matrigel Basement Membrane Matrix (manufactured by BD Biosciences), and subcutaneously transplanted at a concentration of 5×10^6 cells/mouse into the dorsal region of 6-week-old BALB/cAJcl-nu/nu ((CLEA Japan, Inc.)). When the day of transplantation is defined as day 0, SH348-1 or SH357-1 was intraperitoneally administered at a dose of 500 μ g/mouse on days 9, 16, 23, and 30. PBS having the same volume (500 μ l) as that of the antibody was intraperitoneally administered as a control. The tumor volume was measured on days 9, 13, 16, 20, 23, 28, 30, 34, and 37 to study the antitumor effect attributed to the antibody administration. As a result, tumor growth was significantly inhibited in the SH348-1- and SH357-1-administered groups compared to in the PBS-administered group (in the tumor volume comparison with the PBS-administered group on day 37, P values for SH348-1 and SH357-1 were both $P < 0.001$; the P values were calculated by Student's t-test). Moreover, the tumor growth inhibitory rate ($= 100 - (\text{average of tumor volumes of the antibody-administered group}) / (\text{average of tumor volumes of the PBS-administered group}) \times 100$) on day 37 was

89.5% for SH348-1 and 84.1% for SH357-1. Their very strong antitumor effects were observed in vivo (Figures 6A and 6B).

Of 14 anti-EPHA2 monoclonal antibodies studied for their antitumor effects on the MDA-MB-231 cell-transplanted mice, only the antibodies SH348-1 and SH357-1 binding to FnIII-C were shown to be effective (Table 1).

These results demonstrated that SH348-1 and SH357-1 are antibodies which recognize the previously unreported epitope (amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing) and exhibit an antitumor effect. Moreover, the region to which SH348-1 or SH357-1 binds was shown to serve as a promising target of anti-tumor monoclonal antibodies targeted for EPHA2.

[Table 1]

Table 1

Antibody	Binding activity to antigen				Inhibitory effect on tumor growth (in vivo)
	rEPHA2-ECD	rFnIII-NC	rFnIII-N	rFnIII-C	
SH 348-1	+	+	-	+	+
SH 357-1	+	+	-	+	+
Ab 57-1	+	+	+	-	-
Ab 65-1	+	-	-	-	-
Ab 96-1	+	-	-	-	-
Ab 100-1	+	-	-	-	-
Ab 105-1	+	-	-	-	-
Ab 106-13	+	-	-	-	-
Ab 136-1	+	-	-	-	-
Ab 148-1	+	-	-	-	-
Ab 151-4	+	-	-	-	-
Ab 230-1	+	+	+	-	-
Ab 373-1	+	-	-	-	-
Ab 382-1	+	-	-	-	-

(Example 5) Identification of SH348-1 and SH357-1 antibody genes

To determine the heavy and light chain N-terminal amino acid sequences of the mouse anti-human EPHA2 antibodies SH348-1 and SH357-1, an aliquot of a solution containing the SH348-1 or SH357-1 purified in paragraph 2)-8 was separated by SDS-PAGE. The proteins in the gel thus separated were transferred from the gel to a PVDF membrane (0.45 μ m in pore size; manufactured by Invitrogen Corp.). The PVDF membrane was washed with a washing buffer (25 mM NaCl, 10 mM sodium borate buffer,

pH 8.0), then stained by soaking in a staining solution (50% methanol, 20% acetic acid, 0.05% Coomassie Brilliant Blue) for 5 minutes, and then destained with 90% methanol. Band portions corresponding to the heavy and light chains (heavy chain: the band with smaller mobility, light chain: the band with larger mobility) visualized on the PVDF membrane were excised, and an attempt was made to identify their respective N-terminal amino acid sequences by an automatic Edman method (see Edman, P., et al. (1967) Eur. J. Biochem. 1, 80) using Procise (registered trademark) cLC Protein Sequencer Model 492cLC (Applied Biosystems). For the SH-348-1 heavy chain, the amino acid sequence could not be identified by the method. Therefore, the N-terminal pyroglutamic acid was removed using Pfu Pyroglutamate Aminopeptidase (manufactured by TAKARA BIO INC.), and the same procedure as above was then performed to identify an amino acid sequence starting at the second amino acid from the N-terminus.

As a result, the amino acid sequence (starting at the second amino acid from the N-terminus) of the band corresponding to the SH348-1 heavy chain was

I-Q-L-V-Q-S-G-P (SEQ ID NO: 26 in the sequence listing).

The N-terminal amino acid sequence of the band corresponding to the SH348-1 light chain was

D-V-L-M-T-Q-S-P-L-S-L (SEQ ID NO: 27 in the sequence listing).

The N-terminal amino acid sequence of the band corresponding to the SH357-1 heavy chain was

Q-I-Q-L-V-Q-S-G-P (SEQ ID NO: 28 in the sequence listing).

The N-terminal amino acid sequence of the band corresponding to the SH357-1 light chain was

D-V-L-M-T-Q-T-P-L-S-L-P-V-S-L-G-D-Q-A (SEQ ID NO: 29 in the sequence listing).

These amino acid sequences were compared with antibody amino acid sequence database prepared by Kabat et al. (see 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). As a result, the subtype of the SH348-1 heavy chain (γ 2a chain) was miscellaneous, and the subtype of the light chain was kappa light II. Moreover, the subtype of the SH357-1 heavy chain (γ 2a chain) was determined to be miscellaneous, and the subtype of the light chain was determined to be kappa light II.

Thus, the following oligonucleotide primers were synthesized, which respectively hybridized to the 5'-terminal region of an antibody gene coding region belonging to these mouse subtypes and the 3'-terminal region thereof containing a stop codon (see Kabat et al., *ibid*; Matti Kartinen et al. (1988) 25, 859-865; and Heinrich, G. et al. (1984) J. Exp. Med. 159, p. 417-435):

5'-cagatccagttggtgcagtcctggacct-3' (DB3F1: sequence listing sequence ID No. 30)

5'-aagatatctcatttaccgaggagtcgggagaa-3' (MIG2AEVR1:
sequence listing sequence ID No. 31)

5'-aagaattcatgaagttgcctgtagg-3' (MK19EIF1: sequence
listing sequence ID No. 32)

5'-aagatatcttaacactcattcctgttgaagct-3' (KEVR1:
sequence listing sequence ID No. 33)

To clone cDNAs encoding the SH348-1 and SH357-1 heavy and light chains, mRNA was prepared from the SH348-1- or SH357-1-producing hybridomas using Quick Prep mRNA Purification Kit (manufactured by GE Healthcare Bio-Sciences Corp., #27-9254-01). From each mRNA thus obtained, cDNA encoding each antibody heavy or light chain was amplified using TaKaRa One Step RNA PCR Kit (AMV) (manufactured by TAKARA BIO INC., #RR024A) and the primer set for the heavy chain (combination of DB3F1 and MIG2AEVR1) or the primer set for the light chain (combination of MK19EIF1 and KEVR1). These cDNAs amplified by PCR were cloned using Zero Blunt TOPO PCR Cloning Kit (manufactured by Invitrogen Corp.). Each of the cloned heavy and light chain nucleotide sequences was determined using a gene sequence analyzer ("ABI PRISM 3700 DNA Analyzer; Applied Biosystems" or "Applied Biosystems 3730xl Analyzer; Applied Biosystems"). In the sequencing reaction, GeneAmp 9700 (Applied Biosystems) was used.

The determined nucleotide sequence of the cDNA encoding the SH348-1 heavy chain is represented by SEQ ID NO: 34 in the sequence listing, and the amino acid

sequence thereof is represented by SEQ ID NO: 35. The nucleotide sequence of the cDNA encoding the SH348-1 light chain is represented by SEQ ID NO: 36 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 37 in the sequence listing. The nucleotide sequence of the cDNA encoding the SH357-1 heavy chain is represented by SEQ ID NO: 38 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 39. The nucleotide sequence of the cDNA encoding the SH357-1 light chain is represented by SEQ ID NO: 40 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 41. Sequences represented by nucleotide Nos. 1 to 27 and 1327 to 1350 of SEQ ID NO: 34, a sequence represented by nucleotide Nos. 637 to 660 of SEQ ID NO: 36, sequences represented by nucleotide Nos. 1 to 27 and 1327 to 1350 of SEQ ID NO: 38, and a sequence represented by nucleotide Nos. 637 to 660 of SEQ ID NO: 40 are sequences derived from the primers.

Moreover, the amino acid sequences of these heavy and light chains were analyzed by comparison with antibody amino acid sequence database prepared by Kabat et al. (see Kabat, E.A., et al. (1991) in "Sequence of Proteins of Immunological Interest Vol. I and II"; U.S. Department of Health and Human Services). As a result, the SH348-1 heavy chain was shown to have an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 35 in the sequence listing as a variable region

and have an amino acid sequence represented by amino acid Nos. 120 to 449 thereof as a constant region. Moreover, the SH348-1 light chain was shown to have an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 37 in the sequence listing as a variable region and have an amino acid sequence represented by amino acid Nos. 113 to 219 thereof as a constant region.

The SH357-1 heavy chain was shown to have an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 39 in the sequence listing as a variable region and have an amino acid sequence represented by amino acid Nos. 120 to 449 thereof as a constant region. Moreover, the SH357-1 light chain was shown to have an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 41 in the sequence listing as a variable region and have an amino acid sequence represented by amino acid Nos. 113 to 219 thereof as a constant region.

The nucleotide sequence encoding the SH348-1 heavy chain variable region is represented by SEQ ID NO: 42 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 43. The nucleotide sequence encoding the SH348-1 heavy chain constant region is represented by SEQ ID NO: 44, and the amino acid sequence thereof is represented by SEQ ID NO: 45. The nucleotide sequence encoding the SH348-1 light chain variable region is represented by SEQ ID NO: 46 in the sequence listing, and the amino acid sequence thereof is represented by SEQ

ID NO: 47. The nucleotide sequence encoding the SH348-1 light chain constant region is represented by SEQ ID NO: 48, and the amino acid sequence thereof is represented by SEQ ID NO: 49. Sequences represented by nucleotide Nos. 1 to 27 of SEQ ID NO: 42, a sequence represented by nucleotide Nos. 970 to 993 of SEQ ID NO: 44, and sequences represented by nucleotide Nos. 301 to 324 of SEQ ID NO: 48, are sequences derived from the primers.

Moreover, the nucleotide sequence encoding the SH357-1 heavy chain variable region is represented by SEQ ID NO: 50 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 51. The nucleotide sequence encoding the SH357-1 heavy chain constant region is represented by SEQ ID NO: 52, and the amino acid sequence thereof is represented by SEQ ID NO: 53. The nucleotide sequence encoding the SH357-1 light chain variable region is represented by SEQ ID NO: 54 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 55. The nucleotide sequence encoding the SH357-1 light chain constant region is represented by SEQ ID NO: 56, and the amino acid sequence thereof is represented by SEQ ID NO: 57. Sequences represented by nucleotide Nos. 1 to 27 of SEQ ID NO: 50, a sequence represented by nucleotide Nos. 970 to 993 of SEQ ID NO: 52, and sequences represented by nucleotide Nos. 301 to 324 of SEQ ID NO: 56 are sequences derived from the primers.

Furthermore, the positions and sequences of CDRs in each of the amino acid sequences of the heavy and light chain variable regions were analyzed and determined by homology comparison with an antibody amino acid sequence database prepared by Kabat et al. (see Kabat, E.A., et al. (1991) *ibid*). According to the document, even different antibodies have framework regions which have an amino acid length almost equal to each other and are observed to have amino acid sequence commonality in the variable regions, if they are in the same subtype. On the other hand, CDRs are specific sequences flanked by these framework regions. Thus, the amino acid sequences of the heavy and light chains were analyzed by comparison with those of the same subtype thereas. As a result, CDRs in the SH348-1 heavy chain were determined to have amino acid sequences represented by amino acid Nos. 26 to 35 of SEQ ID NO: 35 in the sequence listing (CDRH₁), amino acid Nos. 50 to 66 thereof (CDRH₂), and amino acid Nos. 99 to 108 thereof (CDRH₃). CDRs in the SH348-1 light chain were determined to have amino acid sequences represented by amino acid Nos. 24 to 39 of SEQ ID NO: 37 in the sequence listing (CDRL₁), amino acid Nos. 55 to 61 thereof (CDRL₂), and amino acid Nos. 94 to 102 thereof (CDRL₃). CDRs in the SH357-1 heavy chain were determined to have amino acid sequences represented by amino acid Nos. 26 to 35 of SEQ ID NO: 39 in the sequence listing (CDRH₁), amino acid Nos. 50 to 66 thereof (CDRH₂), and amino acid Nos. 99 to 108 thereof (CDRH₃). CDRs in the

SH357-1 light chain were determined to have amino acid sequences represented by amino acid Nos. 24 to 39 of SEQ ID NO: 41 in the sequence listing (CDRL₁), amino acid Nos. 55 to 61 thereof (CDRL₂), and amino acid Nos. 94 to 102 thereof (CDRL₃).

The nucleotide sequence encoding the SH348-1 CDRH₁ is represented by SEQ ID NO: 58 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 59. The nucleotide sequence encoding the SH348-1 CDRH₂ is represented by SEQ ID NO: 60, and the amino acid sequence thereof is represented by SEQ ID NO: 61. The nucleotide sequence encoding the SH348-1 CDRH₃ is represented by SEQ ID NO: 62, and the amino acid sequence thereof is represented by SEQ ID NO: 63. The nucleotide sequence encoding the SH348-1 CDRL₁ is represented by SEQ ID NO: 64, and the amino acid sequence thereof is represented by SEQ ID NO: 65. The nucleotide sequence encoding the SH348-1 CDRL₂ is represented by SEQ ID NO: 66, and the amino acid sequence thereof is represented by SEQ ID NO: 67. The nucleotide sequence encoding the SH348-1 CDRL₃ is represented by SEQ ID NO: 68, and the amino acid sequence thereof is represented by SEQ ID NO: 69. Moreover, the nucleotide sequence encoding the SH357-1 CDRH₁ is represented by SEQ ID NO: 70, and the amino acid sequence thereof is represented by SEQ ID NO: 71. The nucleotide sequence encoding the SH357-1 CDRH₂ is represented by SEQ ID NO: 72, and the amino acid sequence thereof is represented by SEQ ID NO: 73. The

nucleotide sequence encoding the SH357-1 CDRH₃ is represented by SEQ ID NO: 74, and the amino acid sequence thereof is represented by SEQ ID NO: 75. The nucleotide sequence encoding the SH357-1 CDRL₁ is represented by SEQ ID NO: 76, and the amino acid sequence thereof is represented by SEQ ID NO: 77. The nucleotide sequence encoding the SH357-1 CDRL₂ is represented by SEQ ID NO: 78, and the amino acid sequence thereof is represented by SEQ ID NO: 79. The nucleotide sequence encoding the SH357-1 CDRL₃ is represented by SEQ ID NO: 80, and the amino acid sequence thereof is represented by SEQ ID NO: 81.

(Example 6) Binding activity of anti-EPHA2 antibody to EPHA2 extracellular region

A solution of an EPHA2 extracellular region polypeptide (manufactured by R&D Systems, Inc., #3035-A2-100) or bovine serum albumin (in the description below and the figures, abbreviated to "BSA") as a control diluted to 1 µg/ml with PBS was dispensed at 100 µl/well onto an immunoplate (manufactured by Nunc, #442404) and incubated overnight at 4°C to thereby adsorb the protein to the plate.

On the next day, the solution in the wells was removed, and a Block Ace solution (one pouch of Block Ace powder (manufactured by Dainippon Sumitomo Pharma Co., Ltd. (Show Brand Milk Products Co., Ltd.)) was dissolved in 100 ml of ultrapure water) diluted 4 times with PBS

was dispensed at 200 μl /well and incubated at room temperature for 1 hour. The wells were washed twice with Diluting Buffer (PBS, 0.05% (v/v) Tween 20). Then, SH348-1, SH357-1, and Ab96-1 were separately diluted with PBS to a concentration of 1.25×10^{-4} $\mu\text{g/ml}$, 1.25×10^{-3} $\mu\text{g/ml}$, 1.25×10^{-2} $\mu\text{g/ml}$, 1.25×10^{-1} $\mu\text{g/ml}$, 1.25 $\mu\text{g/ml}$, 12.5 $\mu\text{g/ml}$, or 125 $\mu\text{g/ml}$, and the resulting solution (containing 0.05% (v/v) (final concentration) Tween 20) was added at 100 μl /well.

The plate was incubated at room temperature for 1 hour. Then, the solution in the wells was removed, and the wells were washed twice with Diluting Buffer. Goat anti-Mouse IgG, Peroxidase Conjugated (manufactured by Millipore (Chemicon), #AP181P) diluted 1000 times with Diluting Buffer was added at 100 μl /well and incubated at room temperature for 1 hour. The solution in the wells was removed, and the wells were washed twice with Diluting Buffer. Then, a color reaction was performed with stirring by the addition of OPD Color Developing Solution at 100 μl /well. After color development, the color reaction was terminated by the addition of 1 M HCl

at 100 μ l/well. The absorbance at 490 nm was measured using a plate reader (Figure 7).

Figure 7A) shows the results of SH348-1, Figure 7B) shows the results of SH357-1, and Figure 7C) shows the results of Ab96-1. In each graph, the absorbance is indicated in mean $\pm$ standard deviation ($n = 3$). Stronger absorbance represents stronger binding activity. As shown in the graphs, all the antibodies SH348-1, SH357-1, and Ab96-1 exhibited no affinity for BSA, demonstrating that they specifically bind to the EPHA2 extracellular region.

(Example 7) Influence of anti-EPHA2 antibody on ligand binding

An EPHA2 extracellular region polypeptide (manufactured by R&D Systems, Inc., #3035-A2-100)-immobilized immunoplate was prepared according to the method described in Example 6. The immunoplate wells were washed twice with Diluting Buffer. Then, SH348-1, SH357-1, Ab96-1, or Mouse IgG_{2A} Isotype Control (in the description below and the figures, abbreviated to "mIgG2a"; manufactured by R&D Systems, Inc., #MAB003) as an isotype control antibody diluted to 10 μ g/ml or 50 μ g/ml with Diluting Buffer was added at 100 μ l/well. The plate was incubated at room temperature for 1 hour. Then, Recombinant Mouse Ephrin-A1/Fc Chimera (in the description below and the figures, abbreviated to "Ephrin-A1/Fc"; manufactured by R&D Systems, Inc., #602-

A1-200) as a soluble ligand or Recombinant Human IgG₁ Fc (in the description below and the figures, abbreviated to "hG₁Fc"; manufactured by R&D Systems, Inc., #110-HG-100) as a negative control protein for the soluble ligand was added at a final concentration of 1 µg/ml and incubated at room temperature for 1 hour.

Next, according to the method described in Example 6, the solution in the wells was removed, and the wells were washed with Diluting Buffer. Peroxidase AffiniPure Goat Anti-Human IgG Fcγ Fragment Specific (Jackson ImmunoResearch Laboratories, Inc., #109-035-098) was added thereto. A color reaction was performed using OPD Color Developing Solution. The absorbance at 490 nm was measured using a plate reader (Figure 8).

In Figure 8, the absorbance is indicated in mean±standard deviation (n = 3). The antibody Ab96-1 even at a concentration of 10 µg/ml strongly inhibited the binding of the EPHA2 ligand ephrin-A1 to EPHA2. By contrast, the antibodies SH348-1 and SH357-1, even when added at a concentration of 50 µg/ml (five times the concentration of Ab96-1), did not inhibit the binding of Ephrin-A1/Fc to EPHA2. These results demonstrated that the antibodies SH348-1 and SH357-1 do not inhibit the binding of Ephrin-A1/Fc to EPHA2.

(Example 8) Verification of inhibitory activity of anti-EPHA2 antibody against ephrin-A1-dependent phosphorylation of EPHA2 tyrosine residues

8)-1 Preparation of cell lysates

MDA-MB-231 cells suspended in RPMI1640 containing 10% FBS, 50 units/ml penicillin, and 50 μ g/ml streptomycin (hereinafter, abbreviated to "10% FBS-containing RPMI1640 (with antibiotics)") were seeded at 2.5×10^5 cells/well onto a 12-well dish and cultured overnight at 37°C in 5% CO₂. Next, the medium in the wells was discarded, and RPMI1640 was newly added thereto. The cells were further cultured overnight at 37°C in 5% CO₂. Next, the medium in the wells was removed, and only RPMI1640 or RPMI1640 containing the antibody (mIgG2a, SH348-1, or SH357-1) at a concentration of 10 μ g/ml or 50 μ g/ml was added to each well and preincubated at 37°C in 5% CO₂ for 1 hour.

To the thus-preincubated wells supplemented with only RPMI1640, a 1/50 volume of Ephrin-A1/Fc or hG₁Fc (final concentration of 1 μ g/ml) or RPMI1640 with the same volume thereof (in Figure 9, represented by (-)) were added. Moreover, to the wells supplemented with mIgG2a, SH348-1, or SH357-1, a 1/50 volume of Ephrin-A1/Fc (final concentration of 1 μ g/ml) was added.

The dishes were further incubated at 37°C in 5% CO₂ for 15 minutes. After discarding of the supernatants, 1xCell Lysis Buffer (manufactured by Cell Signaling Technology, Inc.) containing 1 mM PMSF (manufactured by

Sigma-Aldrich, Inc.) and Protease Inhibitor Cocktail (manufactured by Sigma-Aldrich, Inc., #P8340) (hereinafter, abbreviated to PPCLB) was added thereto to lyse the cells. The lysates were centrifuged at 15000 rpm for 5 minutes, and the obtained supernatants were used as immunoprecipitation samples. The protein concentrations of the samples were measured using BCA Protein Assay Reagent (manufactured by PIERCE).

8)-2 Detection of phosphorylated state of EPHA2 by immunoprecipitation

25 μ l of a suspension of Protein G magnetic beads (manufactured by NEW ENGLAND BioLabs, Inc.) and 4 μ g of anti-EphA2 Antibody (manufactured by Santa Cruz Biotechnology, Inc., #sc-924) were added per sample, and the mixture was inverted for mixing at 4°C for 2 hours. Next, FBS was added thereto at a final concentration of 10%, and the mixture was further inverted for mixing at 4°C for 30 minutes. Next, the beads were washed with PPCLB. Next, 200 μ g of the cell lysate supernatants prepared in paragraph 8)-1 were added thereto, and the mixtures were inverted for mixing overnight at 4°C.

On the next day, the beads were washed three times with PPCLB. Then, SDS-Sample Buffer (56.3 mM Tris-HCl, pH 6.8, 1.8% (w/v) SDS, 9% glycerol, 0.72 M 2-mercaptoethanol, 0.045 mg/ml bromophenol blue) was added to the beads, and the mixtures were heated at 98°C for 5 minutes. The proteins dissociated from the beads were separated by SDS-PAGE.

The proteins were transferred from the gel to a PVDF membrane (0.45 μm in pore size; manufactured by Millipore). The PVDF membrane was blocked by shaking in sodium azide-free Blocking Solution (one pouch of Block Ace powder was dissolved in 100 ml of ultrapure water, to which Tween 20 was then added at a final concentration of 0.1% (v/v)).

To detect the phosphorylated state of the EPFA2 tyrosine residues, the PVDF membrane was soaked in a solution of Anti-Phosphotyrosine, recombinant 4G10 HRP-conjugate (manufactured by Upstate, #16-184) diluted 10000 times with sodium azide-free Blocking Solution, and reacted at room temperature for 1 hour. The PVDF membrane was washed for 10 minutes three times with TBST (50 mM Tris-HCl, pH 8.0, 138 mM NaCl, 2.7 mM KCl, 0.1% (v/v) Tween 20) and then further washed for 5 minutes three times with H_2O . Signals were detected on a film for chemiluminescence using ECL Plus (manufactured by GE Healthcare Bio-Sciences Corp.).

Next, to detect the immunoprecipitated EPFA2 present on this PVDF membrane, the PVDF membrane after the detection of the phosphorylated state of the EPFA2 tyrosine residues was soaked in Stripping Solution (50 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 100 mM 2-mercaptoethanol) and shaken at 55°C for 30 minutes. Then, the PVDF membrane was soaked in Quenching Solution (TBST containing 1% (v/v) H_2O_2 and 0.1% (w/v) NaN_3) and shaken at room temperature for 20 minutes to remove the antibody

on the PVDF membrane. The PVDF membrane was washed for 10 minutes three times with TBST and blocked in Blocking Solution (one pouch of Block Ace powder was dissolved in 100 ml of ultrapure water, to which Tween 20 and sodium azide were then added at final concentrations of 0.1% (v/v) and 0.02% (w/v), respectively). Then, the PVDF membrane was soaked in a solution of an anti-EphA2 Antibody (manufactured by Santa Cruz Biotechnology, Inc., #sc-924) diluted 4000 times with Blocking Solution, and reacted at room temperature for 1 hour. The PVDF membrane was washed for 10 minutes three times with TBST. Then, the PVDF membrane was soaked in a solution of Anti-rabbit IgG, HRP-linked Antibody (manufactured by Cell Signaling Technology, Inc., #7074) diluted 10000 times with TBST, and reacted at room temperature for 30 minutes. Then, this PVDF membrane was washed for 10 minutes three times with TBST. Then, signals were detected on a film for chemiluminescence using ECL Plus.

The soluble ligand Ephrin-A1/Fc-induced phosphorylation of EPHA2 tyrosine residues was not inhibited by the isotype control antibody mIgG2a but was inhibited in a dose-dependent manner by the addition of the antibodies SH348-1 and SH357-1 (Figure 9).

These results demonstrated that the antibodies SH348-1 and SH357-1 do not inhibit the binding of the ligand ephrin-A1 to EPHA2 but inhibit the ligand-induced phosphorylation of EPHA2 tyrosine residues.

(Example 9) Epitope identification for anti-EPHA2 antibody

Deletion mutants of EPHA2 consisting of a region shown in Figure 10 were prepared, and a region binding to SH348-1 or SH357-1 was determined.

9)-1 Preparation of deletion mutants of EPHA2

To express deletion mutants of EPHA2 as proteins having GST-Tag and His-Tag added to the N-terminus and S-Tag added to the C-terminus, the following primers were synthesized, and gene fragments amplified by a method shown below were cloned into pET-49b (+) (manufactured by Novagen):

5'-attagatccgagcttccgtactgccagtgtc-3' (primer N1:
sequence ID No. 82)

5'-attagatccgcccccaagggtgaggct-3' (primer N2:
sequence ID No. 83)

5'-attagatccggtcacttaccgcaagaaggagga-3' (primer N3:
sequence ID No. 84)

5'-attagatccggtccagggtgcaggcactgacg-3' (primer N4:
sequence ID No. 85)

5'-aattaagcttgccgccaatcacccgccaagtt-3' (primer C1:
sequence ID No. 86)

5'-aattaagcttggtgccagatccctccggggac-3' (primer C2:
sequence ID No. 87)

5'-aattaagcttcaggtaggtggtgtctctggg-3' (primer C3:
sequence ID No. 88)

5'-aattaagcttctcgtacttccacactcggc-3' (primer C4:
sequence ID No. 89)

Each region was amplified by PCR reaction using pcDNA-DEST40-EPHA2 as the template and a primer set: the primers N1 and C1 for a region consisting of an amino acid sequence represented by amino acid Nos. 426 to 540 of SEQ ID NO: 8 in the sequence listing (hereinafter, referred to as "m1"); the primers N1 and C2 for a region consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 thereof (hereinafter, referred to as "m2"); the primers N1 and C3 for a region consisting of an amino acid sequence represented by amino acid Nos. 426 to 504 thereof (hereinafter, referred to as "m3"); the primers N1 and C4 for a region consisting of an amino acid sequence represented by amino acid Nos. 426 to 470 thereof (hereinafter, referred to as "m4"); the primers N2 and C2 for a region consisting of an amino acid sequence represented by amino acid Nos. 439 to 534 thereof (hereinafter, referred to as "m5"); the primers N3 and C2 for a region consisting of an amino acid sequence represented by amino acid Nos. 471 to 534 thereof (hereinafter, referred to as "m6"); the primers N4 and C2 for a region consisting of an amino acid sequence represented by amino acid Nos. 505 to 534 thereof (hereinafter, referred to as "m7"); the primers N2 and C3 for a region consisting of an amino acid sequence represented by amino acid Nos. 439 to 504 thereof (hereinafter, referred to as "m8"); and the primers N2 and C4 for a region consisting of an amino acid sequence represented by amino acid Nos. 439 to 470

thereof (hereinafter, referred to as "m9"). The obtained PCR products were cleaved with BamHI and HindIII and subcloned into the BamHI/HindIII site of pET-49b (+).

9)-2 Expression of deletion mutants of EPHA2

Escherichia coli BL21 (DE3) was transformed with the plasmid DNA constructed in paragraph 9)-1 or pET-49b (+) as a negative control. The obtained transformants were cultured in an LB medium supplemented with 30 µg/ml kanamycin (manufactured by Invitrogen Corp.). Expression of the deletion mutants of EPHA2 was induced using Autoinduction System (manufactured by Novagen). The bacterial cells were collected by centrifugation and washed with PBS. Then, the bacterial cells were lysed with a 2% SDS solution containing 1 mM PMSF and Protease Inhibitor Cocktail (manufactured by Sigma-Aldrich, Inc., #P8340). The supernatant was collected by centrifugation and used in epitope identification.

The expression level of proteins (consisting of pET-49b (+)-derived GST-Tag, His-Tag, and S-Tag and linker portions for connecting them; in the description below and the figures, referred to as "Vec") expressed in the bacterial cell lysate of *Escherichia coli* transformed with pET-49b (+) was estimated by a method described below. Dilution series of the Vec-expressing bacterial cell lysate and dilution series of 6x His Protein Ladder (manufactured by QIAGEN) were separately dissolved in SDS-Sample Buffer and heated at 98°C for 5 minutes. Then, the proteins were separated by SDS-PAGE, and the proteins

in the gel were transferred to a PVDF membrane. The PVDF membrane was blocked in TBST containing 5% BSA. Then, the PVDF membrane was soaked in a solution of Penta-His HRP Conjugate (manufactured by QIAGEN) diluted 1000 times with TBST containing 5% BSA, and reacted at room temperature for 1 hour. This PVDF membrane was washed for 10 minutes three times with TBST. Then, signals were detected using ECL Plus. Signal intensity was compared between the dilution series of the Vec-expressing bacterial cell lysate and the dilution series of 6x His Protein Ladder, and the concentration of the Vec protein contained in the Vec-expressing bacterial cell lysate was estimated from the protein level per band contained in 6x His Protein Ladder. Deletion mutants of EPHA2 with an amount that exhibited reactivity equivalent to that of 20 ng of Vec in Western blotting using S-Tag Monoclonal Antibody (Novagen) were used in the subsequent epitope identification experiment.

9)-3) Epitope identification

The cell lysates containing the deletion mutants of EPHA2 prepared in paragraph 9)-2 were dissolved in SDS-Sample Buffer and heated at 98°C for 5 minutes. The resulting samples were separated by SDS-PAGE, and the proteins in the gels were transferred to PVDF membranes. After the transfer, the PVDF membranes were blocked by shaking in Blocking Solution (one pouch of Block Ace powder was dissolved in 100 ml of ultrapure water, to which Tween 20 and sodium azide were then added at final

concentrations of 0.1% (v/v) and 0.02% (w/v), respectively). Then, these PVDF membranes were reacted overnight at 4°C in Blocking Solution containing 2 µg/ml SH348-1 or SH357-1. These PVDF membranes were washed for 10 minutes three times with TBST and further reacted at room temperature for 30 minutes in a solution of Anti-Mouse Ig, HRP-Linked Whole Ab Sheep diluted 5000 times with TBST. Subsequently, the PVDF membranes were washed for 10 minutes three times with TBST. Then, signals were detected on a film for chemiluminescence using ECL Plus.

Next, the PVDF membranes were soaked in Stripping Solution (50 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 100 mM 2-mercaptoethanol), then shaken at 55°C for 30 minutes, and then washed for 10 minutes three times with TBST. These PVDF membranes were blocked in Blocking Solution and then washed for 10 minutes three times with TBST. The PVDF membranes were soaked in a solution of S-Tag Monoclonal Antibody diluted 10000 times with TBST, and reacted at room temperature for 30 minutes. The PVDF membranes were washed for 10 minutes three times with TBST. Then, the PVDF membranes were soaked in a solution of Anti-Mouse Ig, HRP-Linked Whole Ab Sheep diluted 5000 times with TBST, and reacted at room temperature for 30 minutes. Next, the PVDF membranes were washed for 10 minutes three times with TBST. Then, signals were detected on a film for chemiluminescence using ECL Plus.

As a result, both the antibodies SH348-1 (Figure 11A) and SH357-1 (Figure 11C) exhibited binding

activities only to m1, m2, and m5. Moreover, in this procedure, the deletion mutants of EPHA2 were present in an almost constant amount on each of the PVDF membranes (SH348-1: Figure 11B, SH357-1: Figure 11D). These results demonstrated that the antibodies SH348-1 and SH357-1 bind to a region consisting of an amino acid sequence represented by amino acid Nos. 439 to 534 of SEQ ID NO: 8 in the sequence listing in the EPHA2 amino acid sequence.

(Example 10) Design of humanized antibody

10)-1 Design of humanized antibody of SH348-1

10)-1-1 Molecular modeling of SH348-1 variable regions

The molecular modeling of SH348-1 variable regions was conducted according to homology modeling (Methods in Enzymology, 203, 121-153, (1991)). The primary sequences (three-dimensional structures derived from X-ray crystal structures are available) of human immunoglobulin variable regions registered in Protein Data Bank (Nuc. Acid Res. 35, D301-D303 (2007)) were compared with the SH348-1 variable regions determined in Example 5. As a result, 2JEL and 1A4J were respectively selected as sequences having the highest sequence homology to the SH348-1 light or heavy chain variable regions. The three-dimensional structures of framework regions were prepared based on a "framework model" obtained by combining the coordinates of 2JEL and 1A4J corresponding

to the SH348-1 light and heavy chains. For SH348-1 CDRs, CDRL₁, CDRL₂, CDRL₃, CDRH₁, and CDRH₂ were assigned to clusters 16A, 7A, 9A, 10A, and 10A, respectively, according to the classification of Thornton et al. (J. Mol. Biol., 263, 800-815, (1996)). CDRH₃ was classified in e (9) D according to the H3-rules (FEBS letters 399, 1-8 (1996)). Subsequently, the typical conformation of each CDR was incorporated in the framework model.

Finally, to obtain possible molecular models of the SH348-1 variable regions in terms of energy, an energy calculation was conducted for excluding disadvantageous interatomic contact. These procedures were performed using a commercially available three-dimensional protein structure prediction program Prime and coordinate search program MacroModel (Schrödinger, LLC).

10)-1-2 Design of amino acid sequence of humanized SH348-1

Humanized SH348-1 antibodies were constructed according to CDR grafting (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). Acceptor antibodies were selected based on amino acid homology within the framework regions. The sequences of the SH348-1 framework regions were compared with those of all human frameworks registered in the Kabat Database (Nuc. Acid Res. 29, 205-206 (2001)) involving antibody amino acid sequences. A GSD2B5B10'CL antibody was selected as an acceptor due to their having, at 72%, the highest sequence homology between their framework regions. The amino acid residues of the

framework regions in GSD2B5B10'CL were aligned with the corresponding amino acid residues in SH348-1 to identify positions where different amino acids therebetween were used. The positions of these residues were analyzed using the three-dimensional model of SH348-1 thus constructed. Then, donor residues to be grafted on the acceptor were selected according to the criteria provided by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). Humanized SH348-1 sequences were determined by transferring some selected donor residues to the acceptor antibody GSD2B5B10'CL. As a result, the humanized sequences of two types of light chains and two types of heavy chains were obtained as shown below. Hereinafter, variable and constant regions and CDRs were classified based on the antibody amino acid sequence database prepared by Kabat et al.

10-1-3) Humanization of SH348-1 light chain

10-1-3-1) hSH-348-T1L-type light chain:

A humanized SH348-1 light chain was designed by substituting amino acid Nos. 2 (valine), 3 (leucine), 14 (serine), 15 (leucine), 17 (aspartic acid), 18 (glutamine), 50 (lysine), 79 (arginine), 88 (leucine), 105 (glycine), 109 (leucine), and 114 (alanine) of SH348-1 light chain variable region shown in SEQ ID NO: 37 of the sequence listing with isoleucine, valine, threonine, proline, glutamic acid, proline, glutamine, lysine, valine, glutamine, valine, and threonine, respectively, and was designated as a "hSH-348-T1L-type light chain".

The nucleotide sequence encoding the hSH348-1-T1L-type light chain is represented by SEQ ID NO: 90 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 91. The nucleotide sequence represented by nucleotide Nos. 1 to 60 of SEQ ID NO: 90 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 61 to 402 thereof is a variable region. The nucleotide sequence represented by nucleotide Nos. 403 to 717 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos. 130 to 177 thereof is CDRL₁. The nucleotide sequence represented by nucleotide Nos. 223 to 243 thereof is CDRL₂. The nucleotide sequence represented by nucleotide Nos. 340 to 363 thereof is CDRL₃.

The amino acid sequence represented by amino acid Nos. 1 to 20 of SEQ ID NO: 91 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 21 to 134 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 135 to 239 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 44 to 59 thereof is CDRL₁. The amino acid sequence represented by amino acid Nos. 75 to 81 thereof is CDRL₂. The amino acid sequence represented by amino acid Nos. 114 to 121 thereof is CDRL₃.

Moreover, the nucleotide sequence encoding the hSH348-1-T1L-type light chain CDRL₁ is represented by SEQ ID NO: 92 in the sequence listing, the amino acid

sequence thereof is represented by SEQ ID NO: 93, the nucleotide sequence of CDRL₂ is represented by SEQ ID NO: 94 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 95, the nucleotide sequence of CDRL₃ is represented by SEQ ID NO: 96 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 97.

10-1-3-2) hSH348-T3L-type light chain:

A humanized SH348-1 light chain was designed by substituting amino acid Nos. 14 (serine), 15 (leucine), 17 (aspartic acid), 18 (glutamine), 50 (lysine), 79 (arginine), 88 (leucine), 105 (glycine), 109 (leucine), and 114 (alanine) of SH348-1 light chain variable region shown in SEQ ID NO: 37 of the sequence listing with threonine, proline, glutamic acid, proline, glutamine, lysine, valine, glutamine, valine, and threonine, respectively, and was designated as a "hSH348-1-T3L-type light chain".

The nucleotide sequence encoding the hSH348-1-T3L-type light chain is represented by SEQ ID NO: 98 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 99. The nucleotide sequence represented by nucleotide Nos. 1 to 60 of SEQ ID NO: 98 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 61 to 402 thereof is a variable region. The nucleotide sequence represented by nucleotide Nos. 403 to 717 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos.

130 to 177 thereof is CDRL₁. The nucleotide sequence represented by nucleotide Nos. 223 to 243 thereof is CDRL₂. The nucleotide sequence represented by nucleotide Nos. 340 to 363 thereof is CDRL₃.

The amino acid sequence represented by amino acid Nos. 1 to 20 of SEQ ID NO: 99 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 21 to 134 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 135 to 239 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 44 to 59 thereof is CDRL₁. The amino acid sequence represented by amino acid Nos. 75 to 81 thereof is CDRL₂. The amino acid sequence represented by amino acid Nos. 114 to 121 thereof is CDRL₃.

Moreover, the nucleotide sequence encoding the hSH348-1-T3L-type light chain CDRL₁ is represented by SEQ ID NO: 100 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 101, the nucleotide sequence of CDRL₂ is represented by SEQ ID NO: 102 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 103, the nucleotide sequence of CDRL₃ is represented by SEQ ID NO: 104 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 105.

10-1-4) Humanization of SH348-1 heavy chain

10-1-4-1) hSH348-1-T1H-type heavy chain:

A humanized SH348-1 heavy chain was designed by substituting amino acid Nos. 2 (isoleucine), 9 (proline), 11 (leucine), 16 (glutamic acid), 17 (threonine), 20 (isoleucine), 38 (lysine), 43 (lysine), 46 (lysine), 68 (phenylalanine), 69 (alanine), 70 (phenylalanine), 71 (serine), 72 (leucine), 73 (glutamic acid), 76 (alanine), 80 (phenylalanine), 82 (glutamine), 83 (isoleucine), 84 (asparagine), 85 (asparagine), 87 (lysine), 88 (asparagine), 93 (threonine), 95 (phenylalanine), 114 (threonine), and 115 (leucine) of SH348-1 heavy chain variable region shown in SEQ ID NO: 43 of the sequence listing with valine, alanine, valine, serine, serine, valine, arginine, glutamine, glutamic acid, valine, threonine, isoleucine, threonine, alanine, aspartic acid, threonine, tyrosine, glutamic acid, leucine, serine, serine, arginine, serine, valine, tyrosine, leucine and valine, respectively, and was designated as a "hSH348-1-T1H-type heavy chain".

The nucleotide sequence encoding the hSH348-1-T1H-type heavy chain is represented by SEQ ID NO: 106 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 107. The nucleotide sequence represented by nucleotide Nos. 1 to 57 of SEQ ID NO: 106 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 58 to 414 thereof is a variable region. The nucleotide sequence represented by nucleotide Nos. 415 to 1404 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos.

148 to 162 thereof is CDRH₁. The nucleotide sequence represented by nucleotide Nos. 205 to 255 thereof is CDRH₂. The nucleotide sequence represented by nucleotide Nos. 352 to 381 thereof is CDRL₃.

The amino acid sequence represented by amino acid Nos. 1 to 19 of SEQ ID NO: 107 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 20 to 138 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 139 to 468 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 50 to 54 thereof is CDRH₁. The amino acid sequence represented by amino acid Nos. 69 to 85 thereof is CDRH₂. The amino acid sequence represented by amino acid Nos. 118 to 127 thereof is CDRH₃.

Moreover, the nucleotide sequence encoding the hSH348-1-T1H-type heavy chain CDRH₁ is represented by SEQ ID NO: 108 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 109, the nucleotide sequence of CDRH₂ is represented by SEQ ID NO: 110 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 111, the nucleotide sequence of CDRH₃ is represented by SEQ ID NO: 112 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 113.

10-1-4-2) hSH348-1-T3H-type heavy chain:

A humanized SH348-1 heavy chain was designed by substituting amino acid Nos. 9 (proline), 11 (leucine),

16 (glutamic acid), 17 (threonine), 20 (isoleucine), 38 (lysine), 43 (lysine), 73 (glutamic acid), 76 (alanine), 80 (phenylalanine), 82 (glutamine), 83 (isoleucine), 84 (asparagine), 85 (asparagine), 87 (lysine), 88 (asparagine), 93 (threonine), 95 (phenylalanine), 114 (threonine), and 115 (leucine) of SH348-1 heavy chain variable domains shown in SEQ ID NO: 43 of the sequence listing with alanine, valine, serine, serine, valine, arginine, glutamine, aspartic acid, threonine, tyrosine, glutamic acid, leucine, serine, serine, arginine, serine, valine, tyrosine, leucine and valine, respectively, and was designated as a "hSH348-1-T3H-type heavy chain".

The nucleotide sequence encoding the hSH348-1-T3H-type heavy chain is represented by SEQ ID NO: 114 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 115.(133). The nucleotide sequence represented by nucleotide Nos. 1 to 57 of SEQ ID NO: 114 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 58 to 414 thereof is a variable region. The nucleotide sequence represented by nucleotide Nos. 415 to 1404 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos. 148 to 162 thereof is CDRH₁. The nucleotide sequence represented by nucleotide Nos. 205 to 255 thereof is CDRH₂. The nucleotide sequence represented by nucleotide Nos. 352 to 381 thereof is CDRL₃.

The amino acid sequence represented by amino acid Nos. 1 to 19 of SEQ ID NO: 115 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 20 to 138 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 139 to 468 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 50 to 54 thereof is CDRH₁. The amino acid sequence represented by amino acid Nos. 69 to 85 thereof is CDRH₂. The amino acid sequence represented by amino acid Nos. 118 to 127 thereof is CDRH₃.

Moreover, the nucleotide sequence encoding the hSH348-1-T3H-type heavy chain CDRH₁ is represented by SEQ ID NO: 116 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 117, the nucleotide sequence of CDRH₂ is represented by SEQ ID NO: 118 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 119, the nucleotide sequence of CDRH₃ is represented by SEQ ID NO: 120 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 121.

10)-2 Design of humanized antibody of SH357-1

10)-2-1 Molecular modeling of SH357-1 variable region

2JEL and 1A4J were selected as sequences having the highest sequence homology to the SH357-1 light and heavy chain variable regions, respectively, in the same way as in paragraph 10)-1-1. CDRL₁, CDRL₂, CDRL₃, CDRH₁, and

CDRH₂ were assigned to clusters 16A, 7A, 9A, 10A, and 10A, respectively. CDRH₃ was classified in e(9)D.

10)-2-2 Design of amino acid sequence for humanized SH357-1

A GSD2B5B10'CL antibody was selected as an acceptor in the same way as in paragraph 10)-1-2, and the sequence of humanized SH357-1 was determined. As a result, the humanized sequences of two types of light chains and two types of heavy chains were obtained as shown below.

10-2-3) Humanization of SH357-1 light chain

10-2-3-1) hSH357-1-T1L-type light chain:

A humanized SH357-1 light chain designed by substituting amino acid Nos. 2 (valine), 3 (leucine), 7 (threonine), 14 (serine), 15 (leucine), 17 (aspartic acid), 18 (glutamine), 50 (lysine), 88 (leucine), 105 (glycine), 109 (leucine), and 114 (alanine) of SH357-1 light chain shown in SEQ ID NO: 41 of the sequence listing with isoleucine, valine, serine, threonine, proline, glutamic acid, proline, glutamine, valine, glutamine, valine, and threonine, respectively, was designated as a "hSH357-1-T1L-type light chain".

The nucleotide sequence encoding the hSH357-1-T1L-type light chain is represented by SEQ ID NO: 122 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 123. The nucleotide sequence represented by nucleotide Nos. 1 to 60 of SEQ ID NO: 122 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 61 to 402 thereof is a

variable region. The nucleotide sequence represented by nucleotide Nos. 403 to 717 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos. 130 to 177 thereof is CDRL₁. The nucleotide sequence represented by nucleotide Nos. 223 to 243 thereof is CDRL₂. The nucleotide sequence represented by nucleotide Nos. 340 to 363 thereof is CDRL₃.

The amino acid sequence represented by amino acid Nos. 1 to 20 of SEQ ID NO: 123 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 21 to 134 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 135 to 239 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 44 to 59 thereof is CDRL₁. The amino acid sequence represented by amino acid Nos. 75 to 81 thereof is CDRL₂. The amino acid sequence represented by amino acid Nos. 114 to 121 thereof is CDRL₃.

Moreover, the nucleotide sequence encoding the hSH357-1-T1L-type light chain CDRL₁ is represented by SEQ ID NO: 124 (154) in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 125 (155), the nucleotide sequence of CDRL₂ is represented by SEQ ID NO: 126 (156) in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 127 (157), the nucleotide sequence of CDRL₃ is represented by SEQ ID NO: 128 (158) in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 129 (159).

10-2-3-2) hSH357-1-T3L-type light chain:

A humanized SH357-1 light chain designed by substituting amino acid Nos. 7 (threonine), 14 (serine), 15 (leucine), 17 (aspartic acid), 18 (glutamine), 50 (lysine), 88 (leucine), 105 (glycine), 109 (leucine), and 114 (alanine) of SH357-1 light chain shown in SEQ ID NO: 41 with serine, threonine, proline, glutamic acid, proline, glutamine, valine, glutamine, valine, and threonine, respectively, was designated as a "hSH357-1-T3L-type light chain".

The nucleotide sequence encoding the hSH357-1-T3L-type light chain is represented by SEQ ID NO: 130 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 131. A nucleotide sequence represented by nucleotide Nos. 1 to 60 of SEQ ID NO: 130 is a secretion signal sequence. A nucleotide sequence represented by nucleotide Nos. 61 to 402 thereof is a variable region. A nucleotide sequence represented by nucleotide Nos. 403 to 717 thereof is a constant region. A nucleotide sequence represented by nucleotide Nos. 130 to 177 thereof is CDRL₁. A nucleotide sequence represented by nucleotide Nos. 223 to 243 thereof is CDRL₂. A nucleotide sequence represented by nucleotide Nos. 340 to 363 thereof is CDRL₃.

The amino acid sequence represented by amino acid Nos. 1 to 20 of SEQ ID NO: 131 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 21 to 134 thereof is a

variable region. The amino acid sequence represented by amino acid Nos. 135 to 239 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 44 to 59 thereof is CDRL₁. The amino acid sequence represented by amino acid Nos. 75 to 81 thereof is CDRL₂. The amino acid sequence represented by amino acid Nos. 114 to 121 thereof is CDRL₃.

Moreover, the nucleotide sequence encoding the hSH357-1-T3L-type light chain CDRL₁ is represented by SEQ ID NO: 132 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 133 (183), the nucleotide sequence of CDRL₂ is represented by SEQ ID NO: 134 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 135, the nucleotide sequence of CDRL₃ is represented by SEQ ID NO: 136 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 137.

10-2-4) Humanization of SH357-1 heavy chain

10-2-4-1) hSH357-1-T1H-type heavy chain:

A humanized SH357-1 heavy chain was designed by substituting amino acid Nos. 2 (isoleucine), 9 (proline), 11 (leucine), 16 (glutamic acid), 17 (threonine), 20 (isoleucine), 38 (lysine), 43 (lysine), 46 (lysine), 68 (phenylalanine), 69 (alanine), 70 (phenylalanine), 71 (serine), 72 (leucine), 73 (glutamic acid), 76 (alanine), 82 (glutamine), 83 (isoleucine), 85 (asparagine), 87 (lysine), 88 (asparagine), 93 (serine), 95 (phenylalanine), 114 (threonine), and 115 (leucine) of

SH357-1 heavy chain variable region shown in SEQ ID NO: 51 of the sequence listing with valine, alanine, valine, alanine, serine, valine, arginine, glutamine, glutamic acid, valine, threonine, isoleucine, threonine, alanine, aspartic acid, threonine, glutamic acid, leucine, serine, arginine, serine, valine, tyrosine, leucine and valine, respectively, and was designated as a "hSH357-1-T1H-type heavy chain".

The nucleotide sequence encoding the hSH357-1-T1H-type heavy chain is represented by SEQ ID NO: 138 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 139. The nucleotide sequence represented by nucleotide Nos. 1 to 57 of SEQ ID NO: 138 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 58 to 414 thereof is a variable region. The nucleotide sequence represented by nucleotide Nos. 415 to 1404 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos. 145 to 162 thereof is CDRH₁. The nucleotide sequence represented by nucleotide Nos. 205 to 255 thereof is CDRH₂. The nucleotide sequence represented by nucleotide Nos. 352 to 381 thereof is CDRL₃.

The amino acid sequence represented by amino acid Nos. 1 to 19 of SEQ ID NO: 139 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 20 to 138 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 139 to 468 thereof is a constant region.

The amino acid sequence represented by amino acid Nos. 49 to 54 thereof is CDRH₁. The amino acid sequence represented by amino acid Nos. 69 to 85 thereof is CDRH₂. The amino acid sequence represented by amino acid Nos. 118 to 127 thereof is CDRH₃.

Moreover, the nucleotide sequence encoding the hSH357-1-T1H-type heavy chain CDRH₁ is represented by SEQ ID NO: 140 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 141, the nucleotide sequence of CDRH₂ is represented by SEQ ID NO: 142 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 143, the nucleotide sequence of CDRH₃ is represented by SEQ ID NO: 144 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 145.

10-2-4-2) hSH357-1-T3H-type heavy chain:

A humanized SH357-1 heavy chain was designed by substituting amino acid Nos. 9 (proline), 11 (leucine), 16 (glutamic acid), 17 (threonine), 20 (isoleucine), 38 (lysine), 43 (lysine), 73 (glutamic acid), 76 (alanine), 82 (glutamine), 83 (isoleucine), 85 (asparagine), 87 (lysine), 88 (asparagine), 93 (serine), 95 (phenylalanine), 114 (threonine), and 115 (leucine) of SH357-1 heavy chain variable region shown in SEQ ID NO: 51 with alanine, valine, alanine, serine, valine, arginine, glutamine, aspartic acid, threonine, glutamic acid, leucine, serine, arginine, serine, valine, tyrosine,

leucine and valine, respectively, and was designated as a "hSH357-1-T3H-type heavy chain".

The nucleotide sequence encoding the hSH357-1-T3H-type heavy chain is represented by SEQ ID NO: 146 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 147. The nucleotide sequence represented by nucleotide Nos. 1 to 57 of SEQ ID NO: 146 is a secretion signal sequence. The nucleotide sequence represented by nucleotide Nos. 58 to 414 thereof is a variable region. The nucleotide sequence represented by nucleotide Nos. 415 to 1404 thereof is a constant region. The nucleotide sequence represented by nucleotide Nos. 145 to 162 thereof is CDRH₁. The nucleotide sequence represented by nucleotide Nos. 205 to 255 thereof is CDRH₂. The nucleotide sequence represented by nucleotide Nos. 352 to 381 thereof is CDRL₃.

The amino acid sequence represented by amino acid Nos. 1 to 19 of SEQ ID NO: 147 of the sequence listing is a secretion signal sequence. The amino acid sequence represented by amino acid Nos. 20 to 138 thereof is a variable region. The amino acid sequence represented by amino acid Nos. 139 to 468 thereof is a constant region. The amino acid sequence represented by amino acid Nos. 49 to 54 thereof is CDRH₁. The amino acid sequence represented by amino acid Nos. 69 to 85 thereof is CDRH₂. The amino acid sequence represented by amino acid Nos. 118 to 127 thereof is CDRH₃.

Moreover, the nucleotide sequence encoding the hSH357-1-T3H-type heavy chain CDRH₁ is represented by SEQ ID NO: 148 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 149, the nucleotide sequence of CDRH₂ is represented by SEQ ID NO: 150 in the sequence listing, the amino acid sequence thereof is represented by SEQ ID NO: 151, the nucleotide sequence of CDRH₃ is represented by SEQ ID NO: 152 in the sequence listing, and the amino acid sequence thereof is represented by SEQ ID NO: 153.

(Example 11) Preparation of humanized anti-EPHA2 antibodies

To measure the activities of the humanized SH348-1 and the humanized SH357-1, plasmids having the heavy and light chains of the humanized anti-EPHA2 antibodies obtained in Example 10 were constructed as shown below.

11)-1 Construction of humanized anti-EPHA2 antibody expression vectors

11)-1-1 Preparation of versatile humanized antibody light chain expression vector (pEF6/KCL)

A gene encoding a human antibody light chain signal sequence and a human Ig light chain (κ chain) constant region, described in SEQ ID NO: 154 in the sequence listing, was synthesized (Invitrogen Corp.; artificial gene synthesis service) and cleaved with restriction enzymes NheI and PmeI. The cleaved DNA fragment was inserted into the NheI/PmeI site of a vector pEF6/V5-HisB

(Invitrogen Corp.) to construct a versatile humanized antibody light chain expression vector (pEF6/KCL).

11)-1-2 Preparation of versatile humanized antibody heavy chain expression vector (pEF1/FCCU-1)

A gene encoding a human antibody heavy chain signal sequence and a human IgG1 constant region, described in SEQ ID NO: 155 in the sequence listing, was synthesized (Invitrogen Corp.; artificial gene synthesis service) and cleaved with restriction enzymes NheI and PmeI. The cleaved DNA fragment was inserted into the NheI/PmeI site of a vector pEF1/myc-HisB (Invitrogen Corp.) to construct a versatile humanized antibody H chain expression vector (pEF1/FCCU-1).

11)-1-3 hSH348-1-T1L and hSH348-1-T3L -type light chain expression vectors

Each DNA containing a gene encoding a hSH348-1-T1L or hSH348-1-T3L-type light chain variable region represented by SEQ ID NO: 156 and 157 of the sequence listing, fused with a secretion signal was synthesized (Invitrogen Corp., Artificial Gene Synthesis Service) and digested with restriction enzymes NdeI and BsiWI. The resulting DNA fragments were separately inserted into sites of the versatile vector for humanized antibody light chain expression (pEF6/KCL) digested in advance with restriction enzymes NdeI and BsiWI to thereby construct hSH348-1-T1L and hSH348-1-T3L-type light chain expression vectors. The obtained expression vectors were

designated as "pEF6/KCL/hSH348-1-T1L" and "pEF6/KCL/hSH348-1-T3L", respectively.

11)-1-4 Construction of hSH348-1-T1H and hSH348-1-T3H-type heavy chain expression vectors

Each DNA containing a gene encoding a hSH348-1-T1H or hSH348-1-T3H-type heavy chain variable region represented by SEQ ID NO: 158 and 159, respectively, of the sequence listing was synthesized (Invitrogen Corp., Artificial Gene Synthesis Service) and digested with a restriction enzyme BspI. The resulting DNA fragments were separately inserted into sites of the versatile vector for humanized antibody H chain expression (pEF1/FCCU-1) digested in advance with a restriction enzyme BspI to thereby construct hSH348-1-T1H and hSH348-1-T3H-type heavy chain expression vectors. The obtained expression vectors were designated as "pEF1/FCCU/hSH348-1-T1H " and "pEF1/FCCU/hSH348-1-T3H", respectively.

11)-1-5 Construction of hSH357-1-T1L and hSH357-1-T3L-type light chain expression vectors

Each DNA containing a gene encoding a hSH357-1-T1L or hSH357-1-T3L-type light chain variable region represented by SEQ ID NO: 160 or 161, respectively, of the sequence listing, fused with a secretion signal was synthesized (Invitrogen Corp., Artificial Gene Synthesis Service) and digested with restriction enzymes NdeI and BsiWI. The resulting DNA fragments were separately inserted into sites of the versatile vector for humanized antibody L chain expression (pEF6/KCL) digested in

advance with restriction enzymes NdeI and BsiWI to thereby construct hSH357-1-T1L and hSH357-1-T3L-type light chain expression vectors. The obtained expression vectors were designated as "pEF6/KCL/hSH357-1-T1L" and "pEF6/KCL/hSH357-1-T3L", respectively.

11)-1-6 Construction of hSH357-1-T1H and hSH357-1-T3H-type heavy chain expression vectors

Each DNA containing a gene encoding a hSH357-1-T1H or hSH357-1-T3H-type heavy chain variable region represented by SEQ ID NO: 162 or 163, respectively, of the sequence listing was synthesized (Invitrogen Corp., Artificial Gene Synthesis Service) and digested with a restriction enzyme BlnI. The resulting DNA fragments were separately inserted into sites of the versatile vector for humanized antibody H chain expression (pEF1/FCCU-1) digested in advance with a restriction enzyme BlnI to thereby construct hSH357-1-T1H and hSH357-1-T3H-type heavy chain expression vectors. The obtained expression vectors were designated as "pEF1/FCCU/hSH357-1-T1H" and "pEF1/FCCU/hSH357-1-T3H", respectively.

11)-2 Production of humanized antibody

1.2×10^9 cells of 293 FreeStyle cells at the log growth phase were seeded onto 1.2 L of fresh FreeStyle 293 Expression Medium (Invitrogen Corp.) and shake-cultured (125 rpm) in an incubator at 37°C in 8% CO₂. 12 mg of Polyethyleneimine (Polyscience #24765) was dissolved in 40 mL of an Opti-Pro SFM medium (manufactured by Invitrogen Corp.) and left at room

temperature for 5 minutes. An H chain expression plasmid (0.6 mg) and an L chain expression plasmid (1.8 mg) prepared using a PureLink HiPure Plasmid kit (Invitrogen Corp.) were suspended in 40 mL of an Opti-Pro SFM medium (Invitrogen Corp.). 40 mL of the expression plasmid/OptiPro SFM mixed solution was added to 40 mL of the Polyethyleneimine/OptiPro SFM mixed solution thus left at room temperature for 5 minutes and further left at room temperature for 5 minutes. Next, 80 mL of the Polyethyleneimine/expression plasmid/OptiPro SFM mixed solution was added to the 293 FreeStyle cell suspension, and shake-culture was continued. After 7-day culture at 37°C in 8% CO₂, the culture supernatant was collected.

11)-3 Purification of humanized antibody

The culture supernatant obtained in paragraph 11)-3 was filtered through a Disposable Capsule Filter (Advantec MFS Inc., #CCS-045-E1H) and then purified by Protein A affinity column chromatography. The culture supernatant was applied to MabSelect SuRe HiTrap 1 mL (manufactured by GE Healthcare Bio-sciences Corp.) equilibrated with PBS, and washed with PBS. Next, a 2 M arginine solution (pH 4.0) was applied thereto to collect antibody-containing fractions. The pH was adjusted to 7, and the antibody-containing fractions were applied to a HiPrep Desalting Column (26/10, 50 mL) (GE Healthcare Bio-sciences Corp.) equilibrated with PBS in advance. After replacement with PBS, the antibody-containing

fractions were passed through a 0.2- μ m filter to prepare a purified sample.

The antibody concentration was determined by eluting the antibodies bound to a POROS G 20 μ m Column, PEEK, 4.6 mm $\times$ 100 mm, 1.7 ml (manufactured by Applied Biosystems) and measuring the absorbance (O.D. 280 nm) of the eluate, followed by peak area comparison with a standard (human IgG1).

A humanized antibody SH348-1 obtained by the combination between pEF6/KCL/hSH348-1-T1L and pEF1/FCCU/hSH348-1-T1H was designated as "hSH348-1-T1"; and a humanized antibody SH348-1 obtained by the combination between pEF6/KCL/hSH348-1-T3L and pEF1/FCCU/hSH348-1-T3H was designated as "hSH348-1-T3".

Moreover, a humanized antibody SH357-1 obtained by the combination between pEF6/KCL/hSH357-1-T1L and pEF1/FCCU/hSH357-1-T1H was designated as "hSH357-1-T1"; and a humanized antibody SH357-1 obtained by the combination between pEF6/KCL/hSH357-1-T3L and pEF1/FCCU/hSH357-1-T3H was designated as "hSH357-1-T3".

(Example 12) Confirmation of binding activity of humanized anti-EPHA2 antibody to antigen

The abilities of the antibodies hSH348-1-T1, hSH348-1-T3, hSH357-1-T1, and hSH357-1-T3 to bind to the antigen were confirmed according to the method described in Example 6 except that Peroxidase AffiniPure Goat Anti-Human IgG Fcy Fragment Specific (manufactured by Jackson

ImmunoResearch Laboratories, Inc., #109-035-098) was used as a secondary antibody.

In the graphs of Figures 12A) to 12D), the absorbance is indicated in mean $\pm$ standard deviation ($n = 3$). As a result, all the humanized anti-EPHA2 antibodies hSH348-1-T1, hSH348-1-T3, hSH357-1-T1, and hSH357-1-T3 were confirmed to have binding activity to the EPHA2 extracellular region.

(Example 13) Measurement of competitive inhibitory activity against binding of SH348-1 or SH357-1 to EPHA2

The competitive inhibitory activities of hSH348-1-T1 and hSH348-1-T3 against the binding of SH348-1 to EPHA2 as well as the competitive inhibitory activities of hSH357-1-T1 and hSH357-1-T3 against the binding of SH357-1 to EPHA2 were measured by a method described below.

The mouse monoclonal antibodies SH348-1 and SH357-1 were separately biotinylated using EZ-Link Sulfo-NHS-LC Biotinylation Kit (manufactured by Thermo Fisher Scientific K.K., #21435) according to the protocol included therein (hereinafter, the biotinylated SH348-1 and SH357-1 were referred to as "bSH348-1" and "bSH357-1", respectively). The concentrations of bSH348-1, bSH357-1, and unlabeled antibodies (SH348-1, SH357-1, hSH348-1-T1, hSH348-1-T3, hSH357-1-T1, hSH357-1-T3, and Ab96-1) used in the competitive inhibition experiment were measured using BCA Protein Assay Reagent (manufactured by PIERCE).

An EPHA2 extracellular region polypeptide (manufactured by R&D Systems, Inc., #3035-A2-100) was diluted to 0.5 µg/ml with PBS, then dispensed at 100 µl/well onto an immunoplate (manufactured by Nunc, #442404), and incubated overnight at 4°C to thereby adsorb the protein onto the plate. On the next day, the wells were washed once with Diluting Buffer (PBS, 0.05% (v/v) Tween 20). Then, a Block Ace solution (one pouch of Block Ace powder was dissolved in 100 ml of ultrapure water) diluted 4 times with PBS was dispensed at 200 µl/well and incubated at room temperature for 4 hours. The solution in the wells was removed. Then, mixed solutions of the biotinylated antibodies (5 µg/ml) and various concentrations (0 µg/ml, 1 µg/ml, 5 µg/ml, 25 µg/ml, 50 µg/ml, and 125 µg/ml) of the unlabeled antibodies (solvent: PBS containing 0.05% (v/v) (final concentration) Tween 20) were separately dispensed at 100 µl/well and incubated at room temperature for 1 hour. The wells were washed twice with Diluting Buffer (PBS, 0.05% (v/v) Tween 20). Then, Streptavidin-horseradish Peroxidase Conjugate (manufactured by GE Healthcare Bio-Sciences Corp., #RPN1231V) diluted 500 times with Diluting Buffer was added at 100 µl/well and incubated at room temperature for 1 hour. The solution in the wells was removed, and the wells were washed twice with Diluting Buffer. Then, a color reaction was performed with stirring by the addition of OPD Color Developing Solution at 100 µl/well. After color development, the

color reaction was terminated by the addition of 1 M HCl at 100 μ l/well. The absorbance at 490 nm was measured using a plate reader.

As a result, the absorbance of the wells supplemented with only bSH348-1 or bSH357-1 was 0.780 ± 0.016 and 0.978 ± 0.007 (mean $\pm$ standard deviation ($n = 3$)), respectively.

In the graphs of Figures 13A) and 13B), the absorbance is indicated in mean $\pm$ standard deviation ($n = 3$). The binding of SH348-1 or SH357-1 to EPHA2 was not inhibited by Ab96-1 differing in epitope therefrom. On the other hand, the binding of SH348-1 to EPHA2 was shown to be inhibited by the antibody SH348-1 itself or its humanized antibodies hSH348-1-T1 and hSH348-1-T3 (Figure 13A). Likewise the binding of SH357-1 to EPHA2 was shown to be inhibited by the antibody SH357-1 itself or its humanized antibodies hSH357-1-T1 and hSH357-1-T3 (Figure 13B).

(Example 14) Inhibitory effect of humanized anti-EPHA2 antibody on ephrin-A1-dependent phosphorylation of EPHA2 tyrosine residues

The ability of the humanized anti-EPHA2 antibody to phosphorylate EPHA2 tyrosine residues in an ephrin-A1-dependent manner was examined according to the method described in Example 8. As a result, all the antibodies hSH348-1-T1, hSH348-1-T3, hSH357-1-T1, and hSH357-1-T3 were shown to maintain an activity of inhibiting Ephrin-

A1/Fc-induced phosphorylation of EPHA2 tyrosine residues (Figure 14).

Industrial Applicability

An anti-EPHA2 antibody of the present invention has an antitumor activity. A pharmaceutical composition comprising the anti-EPHA2 antibody can be used as an anticancer agent.

CLAIMS

1. An antibody which recognizes an epitope recognized by an antibody produced by the hybridoma SH348-1 (FERM BP-10836).
2. The antibody according to claim 1, wherein the antibody has the following properties a) to d):
 - a) having no ability to phosphorylate EPHA2 tyrosine residues;
 - b) having an ADCC activity against EPHA2-expressing cells;
 - c) having a CDC activity against EPHA2-expressing cells; and
 - d) having an antitumor activity in vivo.
3. The antibody according to claim 1, wherein the antibody has the following properties a) to e):
 - a) having no ability to phosphorylate EPHA2 tyrosine residues;
 - b) exhibiting an effect of decreasing an EPHA2 protein level;
 - c) having an ADCC activity against EPHA2-expressing cells;
 - d) having a CDC activity against EPHA2-expressing cells; and
 - e) having an antitumor activity in vivo.
4. An antibody which specifically binds to a polypeptide consisting of an amino acid sequence

represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing.

5. An antibody which specifically binds to a polypeptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing and has the following properties

a) to d):

a) having no ability to phosphorylate EPHA2 tyrosine residues;

b) having an ADCC activity against EPHA2-expressing cells;

c) having a CDC activity against EPHA2-expressing cells; and

d) having an antitumor activity in vivo.

6. An antibody which specifically binds to a peptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing and has the following properties a) to e):

a) having no ability to phosphorylate EPHA2 tyrosine residues;

b) exhibiting an effect of decreasing an EPHA2 protein level;

c) having an ADCC activity against EPHA2-expressing cells;

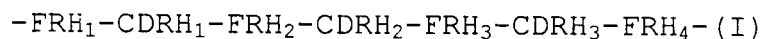
d) having a CDC activity against EPHA2-expressing cells; and

e) having an antitumor activity in vivo.

7. The antibody according to any one of claims 1 to 6, wherein the antibody specifically binds to a peptide consisting of an amino acid sequence represented by amino acid Nos. 439 to 534 of SEQ ID NO: 8 in the sequence listing.
8. The antibody according to any one of claims 1 to 7, wherein the antibody inhibits the phosphorylation of EPHA2 tyrosine residues induced by an EPHA2 ligand.
9. The antibody according to any one of claims 1 to 7, wherein the antibody does not inhibit EPHA2 ligand binding to EPHA2 but inhibits the phosphorylation of EPHA2 tyrosine residues induced by the ligand.
10. An antibody which specifically binds to a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 8 in the sequence listing, wherein the antibody has the amino acid sequences represented by SEQ ID NOs: 59, 61, and 63 in the sequence listing, or amino acid sequences having deletion, substitution, or addition of one or more amino acids in the amino acid sequences, as the complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOs: 65, 67, and 69 in the sequence listing, or amino acid sequences having deletion, substitution, or addition of one or more amino acids in the amino acid sequences, as complementarity determining regions in the light chain variable region.

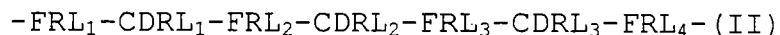
11. An antibody which specifically binds to a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 8 in the sequence listing, characterized by the following 1) and 2):

1) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):



wherein FRH₁ represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids; CDRH₁ represents the amino acid sequence represented by SEQ ID NO: 59 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRH₂ represents an arbitrary amino acid sequence consisting of 14 amino acids; CDRH₂ represents the amino acid sequence represented by SEQ ID NO: 61 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRH₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRH₃ represents the amino acid sequence represented by SEQ ID NO: 63 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; and FRH₄ represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and

2) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):



wherein FRL₁ represents an arbitrary amino acid sequence consisting of 23 amino acids; CDRL₁ represents the amino acid sequence represented by SEQ ID NO: 65 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRL₂ represents an arbitrary amino acid sequence consisting of 15 amino acids; CDRL₂ represents the amino acid sequence represented by SEQ ID NO: 67 in the sequence listing; FRL₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRL₃ represents the amino acid sequence represented by SEQ ID NO: 69 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; and FRL₄ represents an arbitrary amino acid sequence consisting of 10 amino acids, wherein these amino acids are linked to each other through peptide bonds.

12. An antibody which recognizes an epitope recognized by an antibody produced by the hybridoma SH357-1 (FERM BP-10837).

13. The antibody according to claim 12, wherein the antibody has the following properties a) to d):

a) having no ability to phosphorylate EPHA2 tyrosine residues;

b) having an ADCC activity against EPHA2-expressing cells;

c) having a CDC activity against EPHA2-expressing cells; and

d) having an antitumor activity in vivo.

14. An antibody which specifically binds to a peptide consisting of an amino acid sequence represented by amino acid Nos. 426 to 534 of SEQ ID NO: 8 in the sequence listing and has the following properties a) to e):

a) having no ability to phosphorylate EPHA2 tyrosine residues;

b) exhibiting no effect of decreasing an EPHA2 protein level;

c) having an ADCC activity against EPHA2-expressing cells;

d) having a CDC activity against EPHA2-expressing cells; and

e) having an antitumor activity in vivo.

15. The antibody according to any one of claims 12 to 14, wherein the antibody specifically binds to a peptide consisting of an amino acid sequence represented by amino acid Nos. 439 to 534 of SEQ ID NO: 8 in the sequence listing.

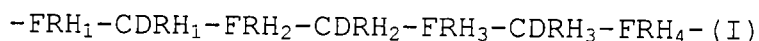
16. The antibody according to any one of claims 12 to 15, wherein the antibody inhibits the phosphorylation of EPHA2 tyrosine residues induced by an EPHA2 ligand.

17. The antibody according to any one of claims 12 to 15, wherein the antibody does not inhibit EPHA2 ligand binding to EPHA2 but inhibits the phosphorylation of EPHA2 tyrosine residues induced by the ligand.

18. An antibody which specifically binds to a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 8 in the sequence listing, wherein the antibody has the amino acid sequences represented by SEQ ID NOs: 71, 73, and 75 in the sequence listing, or amino acid sequences having deletion, substitution, or addition of one or more amino acids in the amino acid sequences, as complementarity determining regions in the heavy chain variable region and has the amino acid sequences represented by SEQ ID NOs: 77, 79, and 81 in the sequence listing, or amino acid sequences having deletion, substitution, or addition of one or more amino acids in the amino acid sequences, as complementarity determining regions in the light chain variable region.

19. An antibody which specifically binds to a polypeptide consisting of the amino acid sequence represented by SEQ ID NO: 8 in the sequence listing, characterized by the following 1) and 2):

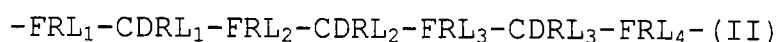
1) having a heavy chain peptide comprising an amino acid sequence represented by the general formula (I):



wherein FRH_1 represents an arbitrary amino acid sequence consisting of 18 to 30 amino acids; CDRH_1 represents the

amino acid sequence represented by SEQ ID NO: 71 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRH₂ represents an arbitrary amino acid sequence consisting of 14 amino acids; CDRH₂ represents the amino acid sequence represented by SEQ ID NO: 73 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRH₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRH₃ represents the amino acid sequence represented by SEQ ID NO: 75 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; and FRH₄ represents an arbitrary amino acid sequence consisting of 11 amino acids, wherein these amino acids are linked to each other through peptide bonds; and

2) having a light chain polypeptide comprising an amino acid sequence represented by the general formula (II):



wherein FRL₁ represents an arbitrary amino acid sequence consisting of 23 amino acids; CDRL₁ represents the amino acid sequence represented by SEQ ID NO: 77 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRL₂ represents an

arbitrary amino acid sequence consisting of 15 amino acids; CDRL₂ represents the amino acid sequence represented by SEQ ID NO: 79 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; FRL₃ represents an arbitrary amino acid sequence consisting of 32 amino acids; CDRL₃ represents the amino acid sequence represented by SEQ ID NO: 81 in the sequence listing or an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence; and FRL₄ represents an arbitrary amino acid sequence consisting of 10 amino acids, wherein these amino acids are linked to each other through peptide bonds.

20. The antibody according to any one of claims 1 to 19, characterized in that the antibody is a humanized antibody.

21. The antibody according to any one of claims 1 to 19, characterized in that the antibody is a human antibody.

22. The antibody according to any one of claims 1 to 19, characterized in that the antibody is an IgG antibody.

23. The antibody according to any one of claims 1 to 9 and 12 to 17, characterized in that the antibody is any selected from Fab, F(ab')₂, Fv, scFv a diabody, a linear antibody, and a multispecific antibody.

24. An antibody produced by the hybridoma SH348-1 (FERM BP-10836).

25. An antibody produced by the hybridoma SH357-1 (FERM BP-10837).

26. An antibody consisting of the following 1) and 2):

1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 35 in the sequence listing or a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 39 in the sequence listing; and

2) a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 37 in the sequence listing or a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 41 in the sequence listing.

27. An antibody consisting of the following 1) or 2):

1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 35 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 37 in the sequence listing; and

2) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 39 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 41 in the sequence listing.

28. An antibody consisting of the following 1) and 2):

1) a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 35 in the sequence listing or a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 39 in the sequence listing; and

2) a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 37 in the sequence listing or a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 41 in the sequence listing.

29. An antibody consisting of the following 1) or 2):

1) a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 35 in the sequence listing and a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 37 in the sequence listing; and

2) a heavy chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 39 in the sequence listing and a light chain polypeptide comprising the amino acid sequence represented by SEQ ID NO: 41 in the sequence listing.

30. An antibody obtained by humanizing an antibody according to any one of claims 24 to 29.

31. An antibody consisting of the following 1) and 2):

1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 107 in the sequence listing or a heavy chain

polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 115 in the sequence listing; and

2) a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 91 in the sequence listing or a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 99 in the sequence listing.

32. An antibody consisting of the following 1) or 2):

1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 107 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 91 in the sequence listing; and

2) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 115 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 99 in the sequence listing.

33. An antibody consisting of the following 1) and 2):

1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 139 in the sequence listing or a heavy chain polypeptide comprising an amino acid sequence represented

by amino acid Nos. 20 to 468 of SEQ ID NO: 147 in the sequence listing; and

2) a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 123 in the sequence listing or a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 131 in the sequence listing.

34. An antibody consisting of the following 1) or 2):

1) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 139 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 123 in the sequence listing; and

2) a heavy chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 147 in the sequence listing and a light chain polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 131 in the sequence listing.

35. A Fab, F(ab')₂, Fv, scFv, a diabody, a linear antibody, or a multispecific antibody derived from any antibodies according to any of claims 24 to 34.

36. Any one polypeptide selected from the group consisting of the following 1) to 20):

1) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 35 in the sequence listing;

2) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 119 of SEQ ID NO: 39 in the sequence listing;

3) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 37 in the sequence listing;

4) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 1 to 112 of SEQ ID NO: 41 in the sequence listing;

5) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 107 in the sequence listing;

6) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 115 in the sequence listing;

7) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 107 in the sequence listing;

8) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 115 in the sequence listing;

9) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 91 in the sequence listing;

10) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 99 in the sequence listing;

11) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 91 in the sequence listing;

12) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 99 in the sequence listing;

13) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 139 in the sequence listing;

14) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 468 of SEQ ID NO: 147 in the sequence listing;

15) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 139 in the sequence listing;

16) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 20 to 138 of SEQ ID NO: 147 in the sequence listing;

17) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 123 in the sequence listing;

18) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 239 of SEQ ID NO: 131 in the sequence listing;

19) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 123 in the sequence listing; and

20) a polypeptide comprising an amino acid sequence represented by amino acid Nos. 21 to 134 of SEQ ID NO: 131 in the sequence listing.

37. The mouse hybridoma SH-348-1 (FERM BP-10836).

38. The mouse hybridoma SH-357-1 (FERM BP-10837).

39. A pharmaceutical composition characterized by comprising at least one antibody selected from the antibodies according to claims 1 to 36.

40. A pharmaceutical composition for cancer treatment characterized by comprising at least one antibody selected from the antibodies according to claims 1 to 36.

41. A method for inhibiting tumor growth in a mammal, comprising administering any antibody selected from the group consisting of antibodies according to claims 1 to 36, 39, and 40.

42. The method for inhibiting tumor growth according to claim 41, characterized in that the tumor is a tumor expressing EPHA2.

43. A polynucleotide encoding an antibody according to any one of claims 1 to 36.

44. A host cell transformed with a polynucleotide according to claim 43.

45. A method for producing an antibody using a host cell according to claim 44.

SEQUENCE LISTING

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<150> JP 2007-224007

<151> 2007-08-30

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Ala Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val
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Trp Ser Phe Gly Ile Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg
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Pro Tyr Trp Glu Leu Ser Asn His Glu Val Met Lys Ala Ile Asn Asp
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Gly Phe Arg Leu Pro Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln
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Leu Met Met Gln Cys Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe
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Ala Asp Ile Val Ser Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser
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Ser Thr Ser Gly Ser Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp
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Gly Tyr Thr Ala Ile Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile
930 935 940

Lys Arg Ile Gly Val Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr
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Glu Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly Trp Asp Leu Met
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Gln Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr Ser Val Cys Asn
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Ile Ala Pro Asp Glu Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His	

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Asp	Leu	Glu	Pro	His	Met	Asn	Tyr	Thr	Phe	Thr	Val	Glu	Ala	Arg	Asn
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Gly Glu Val Tyr Lys Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu
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Val Pro Val Ala Ile Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln
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His Asn Ile Ile Arg Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met
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Met Ile Ile Thr Glu Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu
 Page 15

690

695

700

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Arg Gly Ile Ala Ala Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val
725 730 735

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Cys Lys Val Ser Asp Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro
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Pro Tyr Trp Glu Leu Ser Asn His Glu Val Met Lys Ala Ile Asn Asp
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Gly Phe Arg Leu Pro Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln
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Leu Met Met Gln Cys Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe
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Pro	Ser	Gly	Thr	Phe	Lys	Ala	Asn	Gln	Gly	Asp	Glu	Ala	Cys	Thr	His	
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Cys	Pro	Ile	Asn	Ser	Arg	Thr	Thr	Ser	Glu	Gly	Ala	Thr	Asn	Cys	Val	
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ggc	ctc	aaa	gcc	ggc	gcc	atc	tat	gtc	ttc	cag	gtg	cgg	gca	cgc	acc	1536
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tcg ggc tac acg gag aag cag cgc cgg gac ttc ctg agc gaa gcc tcc Ser Gly Tyr Thr Glu Lys Gln Arg Arg Asp Phe Leu Ser Glu Ala Ser 660 665 670	2016
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Phe Glu Ser Ser Gln Asn Asn Trp Leu Arg Thr Lys Phe Ile Arg Arg
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 Arg Gly Ala His Arg Ile His Val Glu Met Lys Phe Ser Val Arg Asp
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 Cys Ser Ser Ile Pro Ser Val Pro Gly Ser Cys Lys Glu Thr Phe Asn
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 Leu Tyr Tyr Tyr Glu Ala Asp Phe Asp Ser Ala Thr Lys Thr Phe Pro
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 Asn Trp Met Glu Asn Pro Trp Val Lys Val Asp Thr Ile Ala Ala Asp
 130 135 140
 Glu Ser Phe Ser Gln Val Asp Leu Gly Gly Arg Val Met Lys Ile Asn
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 Thr Glu Val Arg Ser Phe Gly Pro Val Ser Arg Ser Gly Phe Tyr Leu
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 Phe Tyr Arg Lys Cys Pro Arg Ile Ile Gln Asn Gly Ala Ile Phe Gln
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 Glu Thr Leu Ser Gly Ala Glu Ser Thr Ser Leu Val Ala Ala Arg Gly
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 Ser Cys Ile Ala Asn Ala Glu Glu Val Asp Val Pro Ile Lys Leu Tyr
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 Cys Asn Gly Asp Gly Glu Trp Leu Val Pro Ile Gly Arg Cys Met Cys
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 Pro Ser Gly Thr Phe Lys Ala Asn Gln Gly Asp Glu Ala Cys Thr His
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785 790 795 800
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820 825 830
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835 840 845
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Gln Ile Gln Leu Val Gln Ser Gly Pro
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Asp Gln Ala

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 aca gtc aag atc tcc tgc aag gct tct ggg tat acc ttc aca cac tat 96
 Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr His Tyr
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 tca atg cac tgg gtg aag cag gct cca gga aag ggt tta aag tgg atg 144
 Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
 35 40 45

 ggc tgg ata aac acc tac act gga gag cca aca tat gct gat gac ttc 192
 Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
 50 55 60

 aag gga cgg ttt gcc ttc tct ttg gaa acc tct gcc agc act gcc ttt 240
 Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Phe
 65 70 75 80

 ttg cag atc aac aac ctc aaa aat gag gac acg gct aca tat ttc tgt 288
 Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys

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Ala	Thr	Tyr	Tyr	Arg	Tyr	Glu	Arg	Asp	Phe	Asp	Tyr	Trp	Gly	Gln	Gly					
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acc	act	ctc	aca	gtc	tcc	tca	gcc	aaa	aca	aca	gcc	cca	tcg	gtc	tat	384				
Thr	Thr	Leu	Thr	Val	Ser	Ser	Ala	Lys	Thr	Thr	Ala	Pro	Ser	Val	Tyr					
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Pro	Leu	Ala	Pro	Val	Cys	Gly	Asp	Thr	Thr	Gly	Ser	Ser	Val	Thr	Leu					
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gga	tgc	ctg	gtc	aag	ggc	tat	ttc	cct	gag	cca	gtg	acc	ttg	acc	tgg	480				
Gly	Cys	Leu	Val	Lys	Gly	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Leu	Thr	Trp					
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aac	tct	gga	tcc	ctg	tcc	agt	ggc	gtg	cac	acc	ttc	cca	gct	gtc	ctg	528				
Asn	Ser	Gly	Ser	Leu	Ser	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu					
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cag	tct	gac	ctc	tac	acc	ctc	agc	agc	tca	gtg	act	gta	acc	tcg	agc	576				
Gln	Ser	Asp	Leu	Tyr	Thr	Leu	Ser	Ser	Ser	Val	Thr	Val	Thr	Ser	Ser					
			180					185					190							
acc	tgg	ccc	agc	cag	tcc	atc	acc	tgc	aat	gtg	gcc	cac	ccg	gca	agc	624				
Thr	Trp	Pro	Ser	Gln	Ser	Ile	Thr	Cys	Asn	Val	Ala	His	Pro	Ala	Ser					
		195					200					205								
agc	acc	aag	gta	gac	aag	aaa	att	gag	ccc	aga	ggg	ccc	aca	atc	aag	672				
Ser	Thr	Lys	Val	Asp	Lys	Lys	Ile	Glu	Pro	Arg	Gly	Pro	Thr	Ile	Lys					
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ccc	tgt	cct	cca	tgc	aaa	tgc	cca	gca	cct	aac	ctc	ttg	ggc	gga	cca	720				
Pro	Cys	Pro	Pro	Cys	Lys	Cys	Pro	Ala	Pro	Asn	Leu	Leu	Gly	Gly	Pro					
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tcc	gtc	ttc	atc	ttc	cct	cca	aag	atc	aag	gat	gta	ctc	atg	atc	tcc	768				
Ser	Val	Phe	Ile	Phe	Pro	Pro	Lys	Ile	Lys	Asp	Val	Leu	Met	Ile	Ser					
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ctg	agc	ccc	ata	gtc	aca	tgt	gtg	gtg	gtg	gat	gtg	agc	gag	gat	gac	816				
Leu	Ser	Pro	Ile	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	Glu	Asp	Asp					
			260					265					270							
cca	gat	gtc	cag	atc	agc	tgg	ttt	gtg	aac	aac	gtg	gaa	gta	cac	aca	864				
Pro	Asp	Val	Gln	Ile	Ser	Trp	Phe	Val	Asn	Asn	Val	Glu	Val	His	Thr					
		275					280					285								
gct	cag	aca	caa	acc	cat	aga	gag	gat	tac	aac	agt	act	ctc	cgg	gtg	912				
Ala	Gln	Thr	Gln	Thr	His	Arg	Glu	Asp	Tyr	Asn	Ser	Thr	Leu	Arg	Val					
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gtc	agt	gcc	ctc	ccc	atc	cag	cac	cag	gac	tgg	atg	agt	ggc	aag	gag	960				
Val	Ser	Ala	Leu	Pro	Ile	Gln	His	Gln	Asp	Trp	Met	Ser	Gly	Lys	Glu					
305					310					315					320					
ttc	aaa	tgc	aag	gtc	aac	aac	aaa	gac	ctc	cca	gcg	ccc	atc	gag	aga	1008				
Phe	Lys	Cys	Lys	Val	Asn	Asn	Lys	Asp	Leu	Pro	Ala	Pro	Ile	Glu	Arg					
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acc	atc	tca	aaa	ccc	aaa	ggg	tca	gta	aga	gct	cca	cag	gta	tat	gtc	1056				
Thr	Ile	Ser	Lys	Pro	Lys	Gly	Ser	Val	Arg	Ala	Pro	Gln	Val	Tyr	Val					
			340					345					350							
ttg	cct	cca	cca	gaa	gaa	gag	atg	act	aag	aaa	cag	gtc	act	ctg	acc	1104				
Leu	Pro	Pro	Pro	Glu	Glu	Glu	Met	Thr	Lys	Lys	Gln	Val	Thr	Leu	Thr					

355	360	365	
tgc atg gtc aca gac ttc atg cct gaa gac att tac gtg gag tgg acc Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr 370 375 380			1152
aac aac ggg aaa aca gag cta aac tac aag aac act gaa cca gtc ctg Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu 385 390 395 400			1200
gac tct gat ggt tct tac ttc atg tac agc aag ctg aga gtg gaa aag Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys 405 410 415			1248
aag aac tgg gtg gaa aga aat agc tac tcc tgt tca gtg gtc cac gag Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu 420 425 430			1296
ggt ctg cac aat cac cac acg act aag agc ttc tcc cgg act ccg ggt Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly 435 440 445			1344
aaa tga Lys			1350

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 <212> PRT
 <213> Mus musculus

<400> 35

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Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr His Tyr
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Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
 35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
 50 55 60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Phe
 65 70 75 80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
 85 90 95

Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly
 100 105 110

Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr
 115 120 125

Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu
 130 135 140

Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp
 145 150 155 160
 Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu
 165 170 175
 Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser
 180 185 190
 Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser
 195 200 205
 Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys
 210 215 220
 Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro
 225 230 235 240
 Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser
 245 250 255
 Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp
 260 265 270
 Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr
 275 280 285
 Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val
 290 295 300
 Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu
 305 310 315 320
 Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg
 325 330 335
 Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val
 340 345 350
 Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr
 355 360 365
 Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr
 370 375 380
 Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu
 385 390 395 400
 Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys
 405 410 415

acc tac agc atg agc agc acc ctc acg ttg acc aag gac gag tat gaa 576
 Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
 180 185 190

cga cat aac agc tat acc tgt gag gcc act cac aag aca tca act tca 624
 Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
 195 200 205

ccc att gtc aag agc ttc aac agg aat gag tgt taa 660
 Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
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<210> 37
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 <212> PRT
 <213> Mus musculus

<400> 37

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Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser
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Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
 85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110

Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
 115 120 125

Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
 130 135 140

Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
 145 150 155 160

Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
 165 170 175

Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
 180 185 190

Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
195 200 205

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<210> 38
<211> 1350
<212> DNA
<213> Mus musculus
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1				5				10					15				
aca gtc aag atc tcc tgc aag gct tct ggg tat acc ttc ata gac tat																	96
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ile Asp Tyr																	
			20					25					30				
tca atg cac tgg gtg aag cag gct cca gga aag ggt tta aag tgg atg																	144
Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met																	
		35					40						45				
ggc tgg ata aac acc tac act gga gag cca aca tat tct gat gac ttc																	192
Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe																	
	50					55					60						
aag gga cgg ttt gcc ttc tct ttg gaa acc tct gcc agc act gcc tat																	240
Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr																	
65					70				75							80	
ttg cag atc agc aac ctc aaa aat gag gac acg gct tca tat ttc tgt																	288
Leu Gln Ile Ser Asn Leu Lys Asn Glu Asp Thr Ala Ser Tyr Phe Cys																	
				85				90						95			
gca acc tac tat agg tac gaa aga gac ttt gac tac tgg ggc caa ggc																	336
Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly																	
			100					105					110				
acc act ctc aca gtc tcc tca gcc aaa aca aca gcc cca tcg gtc tat																	384
Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr																	
			115				120					125					
cca ctg gcc cct gtg tgt gga gat aca act ggc tcc tcg gtg act cta																	432
Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu																	
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gga tgc ctg gtc aag ggt tat ttc cct gag cca gtg acc ttg acc tgg																	480
Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp																	
	145				150				155							160	
aac tct gga tcc ctg tcc agt ggt gtg cac acc ttc cca gct gtc ctg																	528
Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu																	
				165				170						175			
cag tct gac ctc tac acc ctc agc agc tca gtg act gta acc tcg agc																	576
Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Val Thr Ser Ser																	
			180				185						190				

acc tgg ccc agc cag tcc atc acc tgc aat gtg gcc cac ccg gca agc	624
Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser	
195 200 205	
agc acc aag gtg gac aag aaa att gag ccc aga ggg ccc aca atc aag	672
Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys	
210 215 220	
ccc tgt cct cca tgc aaa tgc cca gca cct aac ctc ttg ggt gga cca	720
Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro	
225 230 235 240	
tcc gtc ttc atc ttc cct cca aag atc aag gat gta ctc atg atc tcc	768
Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser	
245 250 255	
ctg agc ccc ata gtc aca tgt gtg gtg gtg gac gtg agc gag gat gac	816
Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp	
260 265 270	
cca gat gtc cag atc agc tgg ttt gtg aac aac gtg gaa gta cac aca	864
Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr	
275 280 285	
gct cag aca caa acc cat aga gag gat tac aac agt act ctc cgg gtg	912
Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val	
290 295 300	
gtc agt gcc ctc ccc atc cag cac cag gac tgg atg agt ggc aag gag	960
Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu	
305 310 315 320	
ttc aaa tgc aag gtc aac aac aaa gac ctc cca gcg ccc atc gag aga	1008
Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg	
325 330 335	
acc atc tca aaa ccc aaa ggg tca gta aga gct cca cag gta tat gtc	1056
Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val	
340 345 350	
ttg cct cca cca gaa gaa gag atg act aag aaa cag gtc act ctg acc	1104
Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr	
355 360 365	
tgc atg gtc aca gac ttc atg cct gaa gac att tac gtg gag tgg acc	1152
Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr	
370 375 380	
aac aac ggg aaa aca gag cta aac tac aag aac act gaa cca gtc ctg	1200
Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu	
385 390 395 400	
gac tct gat ggt tct tac ttc atg tac agc aag ctg aga gtg gaa aag	1248
Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys	
405 410 415	
aag aac tgg gtg gaa aga aat agc tac tcc tgt tca gtg gtc cac gag	1296
Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu	
420 425 430	
ggt ctg cac aat cac cac acg act aag agc ttc tcc cgg act ccg ggt	1344
Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly	
435 440 445	
aaa tga	1350
Lys	

<210> 39
 <211> 449
 <212> PRT
 <213> Mus musculus

<400> 39

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Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
 35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe
 50 55 60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
 65 70 75 80

Leu Gln Ile Ser Asn Leu Lys Asn Glu Asp Thr Ala Ser Tyr Phe Cys
 85 90 95

Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly
 100 105 110

Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr
 115 120 125

Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu
 130 135 140

Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp
 145 150 155 160

Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu
 165 170 175

Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser
 180 185 190

Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser
 195 200 205

Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys
 210 215 220

Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro
 225 230 235 240

Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser
 245 250 255
 Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp
 260 265 270
 Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr
 275 280 285
 Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val
 290 295 300
 Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu
 305 310 315 320
 Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg
 325 330 335
 Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val
 340 345 350
 Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr
 355 360 365
 Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr
 370 375 380
 Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu
 385 390 395 400
 Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys
 405 410 415
 Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu
 420 425 430
 Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly
 435 440 445

Lys

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 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(660)

<400> 40
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gat	caa	gcc	tcc	atc	tct	tgc	aga	tct	agt	cag	agc	att	gta	cat	agt	96
Asp	Gln	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Ile	Val	His	Ser	
			20					25					30			
agt	gga	atc	acc	tat	tta	gaa	tgg	tac	ctg	cag	aaa	cca	ggc	cag	tct	144
Ser	Gly	Ile	Thr	Tyr	Leu	Glu	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser	
		35					40					45				
cca	aag	ctc	ctg	atc	tac	aaa	gtt	tcc	aac	cga	ttt	tct	ggg	gtc	cca	192
Pro	Lys	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro	
	50					55					60					
gac	agg	ttc	agt	ggc	agt	gga	tca	ggg	aca	gat	ttc	aca	ctc	aag	atc	240
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile	
65					70					75					80	
agc	aga	gtg	gag	gct	gag	gat	ctg	gga	gtt	tat	tac	tgc	ttt	caa	ggc	288
Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Val	Tyr	Tyr	Cys	Phe	Gln	Gly	
			85						90					95		
tca	cat	gtt	ccg	tac	acg	ttc	gga	ggg	ggg	acc	aag	ctg	gaa	ata	aaa	336
Ser	His	Val	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	
			100					105					110			
cgg	gct	gat	gct	gca	cca	act	gta	tcc	atc	ttc	cca	cca	tcc	agt	gag	384
Arg	Ala	Asp	Ala	Ala	Pro	Thr	Val	Ser	Ile	Phe	Pro	Pro	Ser	Ser	Glu	
		115					120					125				
cag	tta	aca	tct	gga	ggc	gcc	tca	gtc	gtg	tgc	ttc	ttg	aac	aac	ttc	432
Gln	Leu	Thr	Ser	Gly	Gly	Ala	Ser	Val	Val	Cys	Phe	Leu	Asn	Asn	Phe	
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tac	ccc	aaa	gac	atc	aat	gtc	aag	tgg	aag	att	gat	ggc	agt	gaa	cga	480
Tyr	Pro	Lys	Asp	Ile	Asn	Val	Lys	Trp	Lys	Ile	Asp	Gly	Ser	Glu	Arg	
145					150					155					160	
caa	aat	ggc	gtc	ctg	aac	agt	tgg	act	gat	cag	gac	agc	aaa	gac	agc	528
Gln	Asn	Gly	Val	Leu	Asn	Ser	Trp	Thr	Asp	Gln	Asp	Ser	Lys	Asp	Ser	
				165					170					175		
acc	tac	agc	atg	agc	agc	acc	ctc	acg	ttg	acc	aag	gac	gag	tat	gaa	576
Thr	Tyr	Ser	Met	Ser	Ser	Thr	Leu	Thr	Leu	Thr	Lys	Asp	Glu	Tyr	Glu	
			180					185					190			
cga	cat	aac	agc	tat	acc	tgt	gag	gcc	act	cac	aag	aca	tca	act	tca	624
Arg	His	Asn	Ser	Tyr	Thr	Cys	Glu	Ala	Thr	His	Lys	Thr	Ser	Thr	Ser	
		195					200					205				
ccc	att	gtc	aag	agc	ttc	aac	agg	aat	gag	tgt	taa					660
Pro	Ile	Val	Lys	Ser	Phe	Asn	Arg	Asn	Glu	Cys						
	210					215										

<210> 41
 <211> 219
 <212> PRT
 <213> Mus musculus

<400> 41

Asp	Val	Leu	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	Ile	Val	His	Ser	
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	--

20

25

30

Ser Gly Ile Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 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 Leu Gly Val Tyr Tyr Cys Phe Gln Gly
 85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110

Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
 115 120 125

Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
 130 135 140

Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
 145 150 155 160

Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
 165 170 175

Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
 180 185 190

Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
 195 200 205

Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
 210 215

<210> 42
 <211> 357
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(357)

<400> 42
 cag atc cag ttg gtg cag tct gga cct gag ctg aag aag cct gga gag
 Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
 1 5 10 15

48

aca gtc aag atc tcc tgc aag gct tct ggg tat acc ttc aca cac tat
 Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr His Tyr

96

	20	25	30	
tca atg cac tgg gtg aag cag gct cca gga aag ggt tta aag tgg atg				144
Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met	35	40	45	
ggc tgg ata aac acc tac act gga gag cca aca tat gct gat gac ttc				192
Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe	50	55	60	
aag gga cgg ttt gcc ttc tct ttg gaa acc tct gcc agc act gcc ttt				240
Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Phe	65	70	75	80
ttg cag atc aac aac ctc aaa aat gag gac acg gct aca tat ttc tgt				288
Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys	85	90	95	
gca acc tac tat agg tac gaa aga gac ttt gac tac tgg ggc caa ggc				336
Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly	100	105	110	
acc act ctc aca gtc tcc tca				357
Thr Thr Leu Thr Val Ser Ser	115			

<210> 43
 <211> 119
 <212> PRT
 <213> Mus musculus

<400> 43

Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr His Tyr
20 25 30

Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Phe
65 70 75 80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
85 90 95

Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

<210> 44
 <211> 993

<212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(993)

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<400> 44
gcc aaa aca aca gcc cca tcg gtc tat cca ctg gcc cct gtg tgt gga      48
Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly
1 5 10 15

gat aca act ggc tcc tcg gtg act cta gga tgc ctg gtc aag ggt tat      96
Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
20 25 30

ttc cct gag cca gtg acc ttg acc tgg aac tct gga tcc ctg tcc agt      144
Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser
35 40 45

ggg gtg cac acc ttc cca gct gtc ctg cag tct gac ctc tac acc ctc      192
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu
50 55 60

agc agc tca gtg act gta acc tcg agc acc tgg ccc agc cag tcc atc      240
Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile
65 70 75 80

acc tgc aat gtg gcc cac ccg gca agc agc acc aag gta gac aag aaa      288
Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
85 90 95

att gag ccc aga ggg ccc aca atc aag ccc tgt cct cca tgc aaa tgc      336
Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys
100 105 110

cca gca cct aac ctc ttg ggt gga cca tcc gtc ttc atc ttc cct cca      384
Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro
115 120 125

aag atc aag gat gta ctc atg atc tcc ctg agc ccc ata gtc aca tgt      432
Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys
130 135 140

gtg gtg gtg gat gtg agc gag gat gac cca gat gtc cag atc agc tgg      480
Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp
145 150 155 160

ttt gtg aac aac gtg gaa gta cac aca gct cag aca caa acc cat aga      528
Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg
165 170 175

gag gat tac aac agt act ctc cgg gtg gtc agt gcc ctc ccc atc cag      576
Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln
180 185 190

cac cag gac tgg atg agt ggc aag gag ttc aaa tgc aag gtc aac aac      624
His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn
195 200 205

aaa gac ctc cca gcg ccc atc gag aga acc atc tca aaa ccc aaa ggg      672
Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly
210 215 220

tca gta aga gct cca cag gta tat gtc ttg cct cca cca gaa gaa gag      720
Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu
225 230 235 240

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atg act aag aaa cag gtc act ctg acc tgc atg gtc aca gac ttc atg	768
Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met	
245 250 255	
cct gaa gac att tac gtg gag tgg acc aac aac ggg aaa aca gag cta	816
Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu	
260 265 270	
aac tac aag aac act gaa cca gtc ctg gac tct gat ggt tct tac ttc	864
Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe	
275 280 285	
atg tac agc aag ctg aga gtg gaa aag aag aac tgg gtg gaa aga aat	912
Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn	
290 295 300	
agc tac tcc tgt tca gtg gtc cac gag ggt ctg cac aat cac cac acg	960
Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr	
305 310 315 320	
act aag agc ttc tcc cgg act ccg ggt aaa tga	993
Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys	
325 330	

<210> 45
 <211> 330
 <212> PRT
 <213> Mus musculus

<400> 45

Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly	
1 5 10 15	
Asp Thr Thr Gly ser ser val Thr Leu Gly Cys Leu Val Lys Gly Tyr	
20 25 30	
Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser	
35 40 45	
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu	
50 55 60	
Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile	
65 70 75 80	
Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys	
85 90 95	
Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys	
100 105 110	
Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro	
115 120 125	
Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys	
130 135 140	

Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp
 145 150 155 160
 Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg
 165 170 175
 Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln
 180 185 190
 His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn
 195 200 205
 Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly
 210 215 220
 Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu
 225 230 235 240
 Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met
 245 250 255
 Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu
 260 265 270
 Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe
 275 280 285
 Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn
 290 295 300
 Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr
 305 310 315 320
 Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
 325 330

<210> 46
 <211> 336
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(336)

<400> 46
 gat gtt ttg atg acc caa agt cca ctc tcc ctg cct gtc agt ctt gga 48
 Asp Val Leu Met Thr Gln Ser Pro Leu Ser Leu Pro Val Ser Leu Gly
 1 5 10 15

gat caa gcc tcc atc tct tgc aga tct agt cag agc att gta cat agt 96
 Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser
 20 25 30

aat gga aac acc tat tta gaa tgg tac ctg cag aaa cca ggc cag tct	144
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser	
35 40 45	
cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca	192
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro	
50 55 60	
gac agg ttc agt ggc agt gga tca ggg aca gat ttc aca ctc agg atc	240
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile	
65 70 75 80	
agc aga gtg gag gct gag gat ctg gga gtt tat tac tgc ttt caa ggt	288
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly	
85 90 95	
tca cat gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa	336
Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys	
100 105 110	

<210> 47
 <211> 112
 <212> PRT
 <213> Mus musculus
 <400> 47

Asp Val Leu Met Thr Gln Ser 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 Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 48
 <211> 324
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(324)

<400> 48	48
cgg gct gat gct gca cca act gta tcc atc ttc cca cca tcc agt gag	

Arg	Ala	Asp	Ala	Ala	Pro	Thr	Val	Ser	Ile	Phe	Pro	Pro	Ser	Ser	Glu	
1				5					10					15		
cag	tta	aca	tct	gga	ggt	gcc	tca	gtc	gtg	tgc	ttc	ttg	aac	aac	ttc	96
Gln	Leu	Thr	Ser	Gly	Gly	Ala	Ser	Val	Val	Cys	Phe	Leu	Asn	Asn	Phe	
			20					25					30			
tac	ccc	aaa	gac	atc	aat	gtc	aag	tgg	aag	att	gat	ggc	agt	gaa	cga	144
Tyr	Pro	Lys	Asp	Ile	Asn	Val	Lys	Trp	Lys	Ile	Asp	Gly	Ser	Glu	Arg	
		35					40					45				
caa	aat	ggc	gtc	ctg	aac	agt	tgg	act	gat	cag	gac	agc	aaa	gac	agc	192
Gln	Asn	Gly	Val	Leu	Asn	Ser	Trp	Thr	Asp	Gln	Asp	Ser	Lys	Asp	Ser	
	50					55					60					
acc	tac	agc	atg	agc	agc	acc	ctc	acg	ttg	acc	aag	gac	gag	tat	gaa	240
Thr	Tyr	Ser	Met	Ser	Ser	Thr	Leu	Thr	Leu	Thr	Lys	Asp	Glu	Tyr	Glu	
65					70				75						80	
cga	cat	aac	agc	tat	acc	tgt	gag	gcc	act	cac	aag	aca	tca	act	tca	288
Arg	His	Asn	Ser	Tyr	Thr	Cys	Glu	Ala	Thr	His	Lys	Thr	Ser	Thr	Ser	
				85					90					95		
ccc	att	gtc	aag	agc	ttc	aac	agg	aat	gag	tgt	taa					324
Pro	Ile	Val	Lys	Ser	Phe	Asn	Arg	Asn	Glu	Cys						
			100					105								

<210> 49
 <211> 107
 <212> PRT
 <213> Mus musculus

<400> 49

Arg	Ala	Asp	Ala	Ala	Pro	Thr	Val	Ser	Ile	Phe	Pro	Pro	Ser	Ser	Glu	
1				5					10					15		
Gln	Leu	Thr	Ser	Gly	Gly	Ala	Ser	Val	Val	Cys	Phe	Leu	Asn	Asn	Phe	
			20					25					30			
Tyr	Pro	Lys	Asp	Ile	Asn	Val	Lys	Trp	Lys	Ile	Asp	Gly	Ser	Glu	Arg	
		35					40					45				
Gln	Asn	Gly	Val	Leu	Asn	Ser	Trp	Thr	Asp	Gln	Asp	Ser	Lys	Asp	Ser	
	50					55					60					
Thr	Tyr	Ser	Met	Ser	Ser	Thr	Leu	Thr	Leu	Thr	Lys	Asp	Glu	Tyr	Glu	
65					70				75						80	
Arg	His	Asn	Ser	Tyr	Thr	Cys	Glu	Ala	Thr	His	Lys	Thr	Ser	Thr	Ser	
				85					90					95		
Pro	Ile	Val	Lys	Ser	Phe	Asn	Arg	Asn	Glu	Cys						
			100					105								

<210> 50
 <211> 357
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(357)

<400> 50
 cag atc cag ttg gtg cag tct gga cct gag ctg aag aag cct gga gag 48
 Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
 1 5 10 15
 aca gtc aag atc tcc tgc aag gct tct ggg tat acc ttc ata gac tat 96
 Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ile Asp Tyr
 20 25 30
 tca atg cac tgg gtg aag cag gct cca gga aag ggt tta aag tgg atg 144
 Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
 35 40 45
 ggc tgg ata aac acc tac act gga gag cca aca tat tct gat gac ttc 192
 Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe
 50 55 60
 aag gga cgg ttt gcc ttc tct ttg gaa acc tct gcc agc act gcc tat 240
 Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
 65 70 75 80
 ttg cag atc agc aac ctc aaa aat gag gac acg gct tca tat ttc tgt 288
 Leu Gln Ile Ser Asn Leu Lys Asn Glu Asp Thr Ala Ser Tyr Phe Cys
 85 90 95
 gca acc tac tat agg tac gaa aga gac ttt gac tac tgg ggc caa ggc 336
 Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly
 100 105 110
 acc act ctc aca gtc tcc tca 357
 Thr Thr Leu Thr Val Ser Ser
 115

<210> 51
 <211> 119
 <212> PRT
 <213> Mus musculus

<400> 51
 Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
 1 5 10 15
 Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ile Asp Tyr
 20 25 30
 Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
 35 40 45
 Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe
 50 55 60
 Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
 65 70 75 80
 Leu Gln Ile Ser Asn Leu Lys Asn Glu Asp Thr Ala Ser Tyr Phe Cys
 85 90 95

Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

<210> 52
<211> 993
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(993)

<400> 52
gcc aaa aca aca gcc cca tcg gtc tat cca ctg gcc cct gtg tgt gga 48
Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly
1 5 10 15
gat aca act ggc tcc tcg gtg act cta gga tgc ctg gtc aag ggt tat 96
Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
20 25 30
ttc cct gag cca gtg acc ttg acc tgg aac tct gga tcc ctg tcc agt 144
Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser
35 40 45
ggg gtg cac acc ttc cca gct gtc ctg cag tct gac ctc tac acc ctc 192
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu
50 55 60
agc agc tca gtg act gta acc tcg agc acc tgg ccc agc cag tcc atc 240
Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile
65 70 75 80
acc tgc aat gtg gcc cac ccg gca agc agc acc aag gtg gac aag aaa 288
Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
85 90 95
att gag ccc aga ggg ccc aca atc aag ccc tgt cct cca tgc aaa tgc 336
Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys
100 105 110
cca gca cct aac ctc ttg ggt gga cca tcc gtc ttc atc ttc cct cca 384
Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro
115 120 125
aag atc aag gat gta ctc atg atc tcc ctg agc ccc ata gtc aca tgt 432
Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys
130 135 140
gtg gtc gtg gac gtg agc gag gat gac cca gat gtc cag atc agc tgg 480
Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp
145 150 155 160
ttt gtg aac aac gtg gaa gta cac aca gct cag aca caa acc cat aga 528
Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg
165 170 175
gag gat tac aac agt act ctc cgg gtg gtc agt gcc ctc ccc atc cag 576
Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln
180 185 190

cac	cag	gac	tgg	atg	agt	ggc	aag	gag	ttc	aaa	tgc	aag	gtc	aac	aac	624
His	Gln	Asp	Trp	Met	Ser	Gly	Lys	Glu	Phe	Lys	Cys	Lys	Val	Asn	Asn	
		195					200					205				
aaa	gac	ctc	cca	gcg	ccc	atc	gag	aga	acc	atc	tca	aaa	ccc	aaa	ggg	672
Lys	Asp	Leu	Pro	Ala	Pro	Ile	Glu	Arg	Thr	Ile	Ser	Lys	Pro	Lys	Gly	
	210					215					220					
tca	gta	aga	gct	cca	cag	gta	tat	gtc	ttg	cct	cca	cca	gaa	gaa	gag	720
Ser	Val	Arg	Ala	Pro	Gln	Val	Tyr	Val	Leu	Pro	Pro	Pro	Glu	Glu	Glu	
	225				230					235					240	
atg	act	aag	aaa	cag	gtc	act	ctg	acc	tgc	atg	gtc	aca	gac	ttc	atg	768
Met	Thr	Lys	Lys	Gln	Val	Thr	Leu	Thr	Cys	Met	Val	Thr	Asp	Phe	Met	
				245					250					255		
cct	gaa	gac	att	tac	gtg	gag	tgg	acc	aac	aac	ggg	aaa	aca	gag	cta	816
Pro	Glu	Asp	Ile	Tyr	Val	Glu	Trp	Thr	Asn	Asn	Gly	Lys	Thr	Glu	Leu	
			260					265					270			
aac	tac	aag	aac	act	gaa	cca	gtc	ctg	gac	tct	gat	ggg	tct	tac	ttc	864
Asn	Tyr	Lys	Asn	Thr	Glu	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Tyr	Phe	
		275					280					285				
atg	tac	agc	aag	ctg	aga	gtg	gaa	aag	aag	aac	tgg	gtg	gaa	aga	aat	912
Met	Tyr	Ser	Lys	Leu	Arg	Val	Glu	Lys	Lys	Asn	Trp	Val	Glu	Arg	Asn	
	290					295					300					
agc	tac	tcc	tgt	tca	gtg	gtc	cac	gag	ggg	ctg	cac	aat	cac	cac	acg	960
Ser	Tyr	Ser	Cys	Ser	Val	Val	His	Glu	Gly	Leu	His	Asn	His	His	Thr	
	305				310					315					320	
act	aag	agc	ttc	tcc	cgg	act	ccg	ggg	aaa	tga						993
Thr	Lys	Ser	Phe	Ser	Arg	Thr	Pro	Gly	Lys							
				325					330							

<210> 53
 <211> 330
 <212> PRT
 <213> Mus musculus

<400> 53

Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly
 1 5 10 15

Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
 20 25 30

Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser
 35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu
 50 55 60

Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile
 65 70 75 80

Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
 85 90 95

Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys
100 105 110

Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro
115 120 125

Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys
130 135 140

Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp
145 150 155 160

Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg
165 170 175

Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln
180 185 190

His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn
195 200 205

Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly
210 215 220

Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu
225 230 235 240

Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met
245 250 255

Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu
260 265 270

Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe
275 280 285

Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn
290 295 300

Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr
305 310 315 320

Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
325 330

<210> 54
<211> 336
<212> DNA
<213> Mus musculus

<220>

<221> CDS
 <222> (1)..(336)

<400> 54
 gat gtt ttg atg acc caa act cca ctc tcc ctg cct gtc agt ctt gga 48
 Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
 1 5 10 15
 gat caa gcc tcc atc tct tgc aga tct agt cag agc att gta cat agt 96
 Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser
 20 25 30
 agt gga atc acc tat tta gaa tgg tac ctg cag aaa cca ggc cag tct 144
 Ser Gly Ile Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45
 cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca 192
 Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60
 gac agg ttc agt ggc agt gga tca ggg aca gat ttc aca ctc aag atc 240
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80
 agc aga gtg gag gct gag gat ctg gga gtt tat tac tgc ttt caa ggt 288
 Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
 85 90 95
 tca cat gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa 336
 Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110

<210> 55
 <211> 112
 <212> PRT
 <213> Mus musculus

<400> 55
 Asp Val Leu 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 Ile Val His Ser
 20 25 30
 Ser Gly Ile Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45
 Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 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 Leu Gly Val Tyr Tyr Cys Phe Gln Gly
 85 90 95
 Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110

<210> 56
 <211> 324
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(324)

<400> 56
 cgg gct gat gct gca cca act gta tcc atc ttc cca cca tcc agt gag 48
 Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
 1 5 10 15
 cag tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg aac aac ttc 96
 Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
 20 25 30
 tac ccc aaa gac atc aat gtc aag tgg aag att gat ggc agt gaa cga 144
 Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
 35 40 45
 caa aat ggc gtc ctg aac agt tgg act gat cag gac agc aaa gac agc 192
 Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
 50 55 60
 acc tac agc atg agc agc acc ctc acg ttg acc aag gac gag tat gaa 240
 Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
 65 70 75 80
 cga cat aac agc tat acc tgt gag gcc act cac aag aca tca act tca 288
 Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
 85 90 95
 ccc att gtc aag agc ttc aac agg aat gag tgt taa 324
 Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
 100 105

<210> 57
 <211> 107
 <212> PRT
 <213> Mus musculus

<400> 57
 Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
 1 5 10 15
 Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
 20 25 30
 Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
 35 40 45
 Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
 50 55 60
 Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
 65 70 75 80
 Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser

85

90

95

Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
 100 105

<210> 58
 <211> 30
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(30)

<400> 58
 ggg tat acc ttc aca cac tat tca atg cac 30
 Gly Tyr Thr Phe Thr His Tyr Ser Met His
 1 5 10

<210> 59
 <211> 10
 <212> PRT
 <213> Mus musculus

<400> 59
 Gly Tyr Thr Phe Thr His Tyr Ser Met His
 1 5 10

<210> 60
 <211> 51
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(51)

<400> 60
 tgg ata aac acc tac act gga gag cca aca tat gct gat gac ttc aag 48
 Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
 1 5 10 15

gga 51
 Gly

<210> 61
 <211> 17
 <212> PRT
 <213> Mus musculus

<400> 61
 Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
 1 5 10 15

Gly

<210> 62
 <211> 30
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(30)

<400> 62
 tac tat agg tac gaa aga gac ttt gac tac 30
 Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

<210> 63
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 <212> PRT
 <213> Mus musculus

<400> 63
 Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

<210> 64
 <211> 48
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(48)

<400> 64
 aga tct agt cag agc att gta cat agt aat gga aac acc tat tta gaa 48
 Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
 1 5 10 15

<210> 65
 <211> 16
 <212> PRT
 <213> Mus musculus

<400> 65
 Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
 1 5 10 15

<210> 66
 <211> 21
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(21)

<400> 66
 aaa gtt tcc aac cga ttt tct 21
 Lys Val Ser Asn Arg Phe Ser

1 5

<210> 67
<211> 7
<212> PRT
<213> Mus musculus

<400> 67

Lys Val Ser Asn Arg Phe Ser
1 5

<210> 68
<211> 27
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(27)

<400> 68
ttt caa ggt tca cat gtt ccg tac acg
Phe Gln Gly Ser His Val Pro Tyr Thr
1 5

27

<210> 69
<211> 9
<212> PRT
<213> Mus musculus

<400> 69

Phe Gln Gly Ser His Val Pro Tyr Thr
1 5

<210> 70
<211> 30
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(30)

<400> 70
ggg tat acc ttc ata gac tat tca atg cac
Gly Tyr Thr Phe Ile Asp Tyr Ser Met His
1 5 10

30

<210> 71
<211> 10
<212> PRT
<213> Mus musculus

<400> 71

Gly Tyr Thr Phe Ile Asp Tyr Ser Met His
1 5 10

<210> 72
 <211> 51
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(51)

<400> 72
 tgg ata aac acc tac act gga gag cca aca tat tct gat gac ttc aag 48
 Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe Lys
 1 5 10 15
 gga 51
 Gly

<210> 73
 <211> 17
 <212> PRT
 <213> Mus musculus

<400> 73
 Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe Lys
 1 5 10 15
 Gly

<210> 74
 <211> 30
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(30)

<400> 74
 tac tat agg tac gaa aga gac ttt gac tac 30
 Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

<210> 75
 <211> 10
 <212> PRT
 <213> Mus musculus

<400> 75
 Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

<210> 76
 <211> 48
 <212> DNA
 <213> Mus musculus

<220>
<221> CDS
<222> (1)..(48)

<400> 76
aga tct agt cag agc att gta cat agt agt gga atc acc tat tta gaa 48
Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu
1 5 10 15

<210> 77
<211> 16
<212> PRT
<213> Mus musculus

<400> 77
Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu
1 5 10 15

<210> 78
<211> 21
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(21)

<400> 78
aaa gtt tcc aac cga ttt tct 21
Lys Val Ser Asn Arg Phe Ser
1 5

<210> 79
<211> 7
<212> PRT
<213> Mus musculus

<400> 79
Lys Val Ser Asn Arg Phe Ser
1 5

<210> 80
<211> 27
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(27)

<400> 80
ttt caa ggt tca cat gtt ccg tac acg 27
Phe Gln Gly Ser His Val Pro Tyr Thr
1 5

<210> 81
<211> 9
<212> PRT
<213> Mus musculus

<400> 81

Phe Gln Gly Ser His Val Pro Tyr Thr
1 5

<210> 82

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> primer N1

<400> 82

attaggatcc gagcttccgt actgccagtg tc

32

<210> 83

<211> 28

<212> DNA

<213> Artificial

<220>

<223> primer N2

<400> 83

attaggatcc gccccccaag gtgaggct

28

<210> 84

<211> 34

<212> DNA

<213> Artificial

<220>

<223> primer N3

<400> 84

attaggatcc ggtcacttac cgcaagaagg gaga

34

<210> 85

<211> 32

<212> DNA

<213> Artificial

<220>

<223> primer N4

<400> 85

attaggatcc ggtccagggtg caggcactga cg

32

<210> 86

<211> 31

<212> DNA

<213> Artificial

<220>

<223> primer C1

<400> 86

aattaagctt gccgccaatc accgccaagt t

31

<210> 87

<211> 32
 <212> DNA
 <213> Artificial

 <220>
 <223> primer C2

 <400> 87
 aattaagctt gttgccagat ccctccgggg ac 32

<210> 88
 <211> 29
 <212> DNA
 <213> Artificial

 <220>
 <223> primer C3

 <400> 88
 aattaagctt caggtagggtg gtgtctggg 29

<210> 89
 <211> 30
 <212> DNA
 <213> Artificial

 <220>
 <223> primer C4

 <400> 89
 aattaagctt ctcgtacttc cacactcggc 30

<210> 90
 <211> 720
 <212> DNA
 <213> Artificial

 <220>
 <223> hSH-348-T1L

<220>
 <221> CDS
 <222> (1)..(720)

 <220>
 <221> sig_peptide
 <222> (1)..(60)

<400> 90
 atg gtg ctg cag acc cag gtg ttc atc tcc ctg ctg ctg tgg atc tcc 48
 Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser
 1 5 10 15

ggc gca tat ggc gac ata gtg atg acc cag agc ccc ctg agc ctg ccc 96
 Gly Ala Tyr Gly Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro
 20 25 30

gtg acc ccc ggc gag ccc gcc tcc atc agc tgc cgg agc agc cag agc 144
 Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
 35 40 45

atc gtg cac agc aac ggc aac acc tac ctg gag tgg tac ctg cag aag 192
 Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys
 50 55 60

ccc ggc cag agc ccc cag ctg ctg atc tac aag gtg agc aac cgg ttc Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe 65 70 75 80	240
agc ggc gtg ccc gac cgg ttc agc ggc agc ggc acc gac ttc Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe 85 90 95	288
acc ctg aag atc agc cgg gtg gag gcc gag gac gtg ggc gtg tac tac Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr 100 105 110	336
tgc ttc cag ggc tcc cac gtg ccc tac acc ttc ggc cag ggc acc aag Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys 115 120 125	384
gtg gag atc aag cgt acg gtg gcc gcc ccc tcc gtg ttc atc ttc ccc Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro 130 135 140	432
ccc tcc gac gag cag ctg aag tcc ggc acc gcc tcc gtg gtg tgc ctg Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu 145 150 155 160	480
ctg aat aac ttc tac ccc aga gag gcc aag gtg cag tgg aag gtg gac Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp 165 170 175	528
aac gcc ctg cag tcc ggg aac tcc cag gag agc gtg acc gag cag gac Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp 180 185 190	576
agc aag gac agc acc tac agc ctg agc agc acc ctg acc ctg agc aaa Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys 195 200 205	624
gcc gac tac gag aag cac aag gtg tac gcc tgc gag gtg acc cac cag Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln 210 215 220	672
ggc ctg agc tcc ccc gtc acc aag agc ttc aac agg ggc gag tgt tag Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys 225 230 235	720

<210> 91
 <211> 239
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 91

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 Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro
 20 25 30

Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
 35 40 45

Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys
 50 55 60
 Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys 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 Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys
 115 120 125
 Val Glu 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
 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> 92
 <211> 48
 <212> DNA
 <213> Artificial

<220>
 <223> hSH348-1-T1L CDRL1

<220>
 <221> CDS
 <222> (1)..(48)

<400> 92
 cgg agc agc cag agc atc gtc cac agc aac ggc aac acc tac ctg gag
 Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
 1 5 10 15

48

<210> 93

<211> 16
 <212> PRT
 <213> Artificial

 <220>
 <223> Synthetic Construct

 <400> 93
 Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
 1 5 10 15

<210> 94
 <211> 21
 <212> DNA
 <213> Artificial

 <220>
 <223> hSH348-1-T1L CDRL2

<220>
 <221> CDS
 <222> (1)..(21)

<400> 94
 aag gtg agc aac cgg ttc agc
 Lys Val Ser Asn Arg Phe Ser
 1 5

21

<210> 95
 <211> 7
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

 <400> 95

Lys Val Ser Asn Arg Phe Ser
 1 5

<210> 96
 <211> 24
 <212> DNA
 <213> Artificial

<220>
 <223> hSH348-1-T1L CDRL3

<220>
 <221> CDS
 <222> (1)..(24)

<400> 96
 ttc cag ggc tcc cac gtg ccc tac
 Phe Gln Gly Ser His Val Pro Tyr
 1 5

24

<210> 97
 <211> 8
 <212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 97

Phe Gln Gly Ser His Val Pro Tyr
1 5

<210> 98

<211> 720

<212> DNA

<213> Artificial

<220>

<223> hSH348-1-T3L

<220>

<221> CDS

<222> (1)..(720)

<220>

<221> sig_peptide

<222> (1)..(60)

<400> 98

atg	gtg	ctg	cag	acc	cag	gtg	ttc	atc	tcc	ctg	ctg	ctg	tgg	atc	tcc	48
Met	Val	Leu	Gln	Thr	Gln	Val	Phe	Ile	Ser	Leu	Leu	Leu	Trp	Ile	Ser	
1				5					10					15		

ggc	gca	tat	ggc	gac	gtg	ctg	atg	acc	cag	agc	ccc	ctg	agc	ctg	ccc	96
Gly	Ala	Tyr	Gly	Asp	Val	Leu	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	
			20					25					30			

gtg	acc	ccc	ggc	gag	ccc	gcc	tcc	atc	agc	tgc	cgg	agc	agc	cag	agc	144
Val	Thr	Pro	Gly	Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	
		35				40						45				

atc	gtg	cac	agc	aac	ggc	aac	acc	tac	ctg	gag	tgg	tac	ctg	cag	aag	192
Ile	Val	His	Ser	Asn	Gly	Asn	Thr	Tyr	Leu	Glu	Trp	Tyr	Leu	Gln	Lys	
	50					55					60					

ccc	ggc	cag	agc	ccc	cag	ctg	ctg	atc	tac	aag	gtg	agc	aac	cgg	ttc	240
Pro	Gly	Gln	Ser	Pro	Gln	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	
65					70					75					80	

agc	ggc	gtg	ccc	gac	cgg	ttc	agc	ggc	agc	ggc	agc	ggc	acc	gac	ttc	288
Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	
				85				90						95		

acc	ctg	aag	atc	agc	cgg	gtg	gag	gcc	gag	gac	gtg	ggc	gtg	tac	tac	336
Thr	Leu	Lys	Ile	Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	
			100					105					110			

tgc	ttc	cag	ggc	tcc	cac	gtg	ccc	tac	acc	ttc	ggc	cag	ggc	acc	aag	384
Cys	Phe	Gln	Gly	Ser	His	Val	Pro	Tyr	Thr	Phe	Gly	Gln	Gly	Thr	Lys	
		115					120					125				

gtg	gag	atc	aag	cgt	acg	gtg	gcc	gcc	ccc	tcc	gtg	ttc	atc	ttc	ccc	432
Val	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	
	130					135					140					

ccc	tcc	gac	gag	cag	ctg	aag	tcc	ggc	acc	gcc	tcc	gtg	gtg	tgc	ctg	480
Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	
145					150					155					160	

ctg aat aac ttc tac ccc aga gag gcc aag gtg cag tgg aag gtg gac 528
 Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
 165 170 175

aac gcc ctg cag tcc ggg aac tcc cag gag agc gtg acc gag cag gac 576
 Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
 180 185 190

agc aag gac agc acc tac agc ctg agc agc acc ctg acc ctg agc aaa 624
 Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
 195 200 205

gcc gac tac gag aag cac aag gtg tac gcc tgc gag gtg acc cac cag 672
 Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
 210 215 220

ggc ctg agc tcc ccc gtc acc aag agc ttc aac agg ggg gag tgt tag 720
 Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 225 230 235

<210> 99
 <211> 239
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 99

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 Val Leu Met Thr Gln Ser Pro Leu Ser Leu Pro
 20 25 30

Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
 35 40 45

Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys
 50 55 60

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys 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 Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys
 115 120 125

Val Glu 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
 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> 100
 <211> 48
 <212> DNA
 <213> Artificial

<220>
 <223> hSH348-1-T3L CDRL1

<220>
 <221> CDS
 <222> (1)..(48)

<400> 100
 cgg agc agc cag agc atc gtg cac agc aac ggc aac acc tac ctg gag 48
 Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
 1 5 10 15

<210> 101
 <211> 16
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 101

Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
 1 5 10 15

<210> 102
 <211> 21
 <212> DNA
 <213> Artificial

<220>
 <223> hSH348-1-T3L CDRL2

<220>
 <221> CDS

<222> (1)..(21)

<400> 102

aag gtg agc aac cgg ttc agc
Lys Val Ser Asn Arg Phe Ser
1 5

21

<210> 103

<211> 7

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 103

Lys Val Ser Asn Arg Phe Ser
1 5

<210> 104

<211> 24

<212> DNA

<213> Artificial

<220>

<223> hSH348-1-T3L CDRL3

<220>

<221> CDS

<222> (1)..(24)

<400> 104

ttc cag ggc tcc cac gtg ccc tac
Phe Gln Gly Ser His Val Pro Tyr
1 5

24

<210> 105

<211> 8

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 105

Phe Gln Gly Ser His Val Pro Tyr
1 5

<210> 106

<211> 1407

<212> DNA

<213> Artificial

<220>

<223> hSH348-1-T1H

<220>

<221> CDS

<222> (1)..(1407)

<220>
 <221> sig_peptide
 <222> (1)..(57)

<400> 106
 atg aaa cac ctg tgg ttc ttc ctc ctg ctg gtg gca gct ccc aga tgg 48
 Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
 1 5 10 15
 gtg ctg agc cag gtg cag ctg gtg cag agc ggc gcc gag gtg aag aag 96
 Val Leu Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
 20 25 30
 ccc ggc agc agc gtg aag gtg agc tgc aag gcc agc ggc tac acc ttc 144
 Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45
 acc cac tac agc atg cac tgg gtg cgg cag gcc ccc ggc cag ggc ctg 192
 Thr His Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
 50 55 60
 gag tgg atg ggc tgg atc aac acc tac acc ggc gag ccc acc tac gcc 240
 Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala
 65 70 75 80
 gac gac ttc aag ggc cgg gtg acc atc acc gcc gac acc tcc acc tcc 288
 Asp Asp Phe Lys Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser
 85 90 95
 acc gcc tac ctg gaa ctg agc agc ctg cgg agc gag gac acc gcc gtg 336
 Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
 100 105 110
 tac tac tgc gcc acc tac tac cgg tac gag cgg gac ttc gac tac tgg 384
 Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp
 115 120 125
 ggc cag ggc acc ctg gtg acc gtg agc tca gcc tcc acc aag ggc cca 432
 Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 130 135 140
 agc gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggc ggc aca 480
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 145 150 155 160
 gcc gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa ccc gtg acc 528
 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 165 170 175
 gtg agc tgg aac tca ggc gcc ctg acc agc ggc gtg cac acc ttc ccc 576
 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 180 185 190
 gct gtc ctg cag tcc tca gga ctc tac tcc ctc agc agc gtg gtg acc 624
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 195 200 205
 gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac gtg aat 672
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 210 215 220
 cac aag ccc agc aac acc aag gtg gac aag aga gtt gag ccc aaa tct 720
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
 225 230 235 240
 tgt gac aaa act cac aca tgc cca ccc tgc cca gca cct gaa ctc ctg 768
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 245 250 255

ggg gga ccc tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu 260 265 270	816
atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser 275 280 285	864
cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu 290 295 300	912
gtg cat aat gcc aag aca aag ccc cgg gag gag cag tac aac agc acg Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr 305 310 315 320	960
tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn 325 330 335	1008
ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro 340 345 350	1056
atc gag aaa acc atc tcc aaa gcc aaa ggc cag ccc cgg gaa cca cag Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln 355 360 365	1104
gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val 370 375 380	1152
agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val 385 390 395 400	1200
gag tgg gag agc aat ggc cag ccc gag aac aac tac aag acc acc cct Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro 405 410 415	1248
ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr 420 425 430	1296
gtg gac aag agc agg tgg cag cag ggc aac gtc ttc tca tgc tcc gtg Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val 435 440 445	1344
atg cat gag gct ctg cac aac cac tac acc cag aag agc ctc tcc ctg Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu 450 455 460	1392
tct ccc ggc aaa tga Ser Pro Gly Lys 465	1407

<210> 107
 <211> 468
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 107

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
 1 5 10 15
 Val Leu Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
 20 25 30
 Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45
 Thr His Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
 50 55 60
 Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala
 65 70 75 80
 Asp Asp Phe Lys Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser
 85 90 95
 Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
 100 105 110
 Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp
 115 120 125
 Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 130 135 140
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 145 150 155 160
 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 165 170 175
 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 180 185 190
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 195 200 205
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 210 215 220
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
 225 230 235 240
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 245 250 255
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 260 265 270

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
275 280 285

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
290 295 300

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
305 310 315 320

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
325 330 335

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
340 345 350

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
355 360 365

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
370 375 380

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
385 390 395 400

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
405 410 415

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
420 425 430

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
435 440 445

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
450 455 460

Ser Pro Gly Lys
465

<210> 108
<211> 15
<212> DNA
<213> Artificial

<220>
<223> hSH348-1-T1H CDRH1

<220>
<221> CDS
<222> (1)..(15)

<400> 108
cac tac agc atg cac
His Tyr Ser Met His

1 5

<210> 109
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 109

His Tyr Ser Met His
1 5

<210> 110
<211> 51
<212> DNA
<213> Artificial

<220>
<223> hSH348-1-T1H_CDRH2

<220>
<221> CDS
<222> (1)..(51)

<400> 110
tgg atc aac acc tac acc ggc gag ccc acc tac gcc gac gac ttc aag 48
Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
1 5 10 15

ggc 51
Gly

<210> 111
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 111

Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
1 5 10 15

Gly

<210> 112
<211> 30
<212> DNA
<213> Artificial

<220>
<223> hSH348-1-T1H_CDRH3

<220>

<221> CDS
 <222> (1)..(30)

 <400> 112
 tac tac cgg tac gag cgg gac ttc gac tac 30
 Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

 <210> 113
 <211> 10
 <212> PRT
 <213> Artificial

 <220>
 <223> Synthetic Construct

 <400> 113
 Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

 <210> 114
 <211> 1407
 <212> DNA
 <213> Artificial

 <220>
 <223> hSH348-1-T3H

 <220>
 <221> CDS
 <222> (1)..(1407)

 <220>
 <221> sig_peptide
 <222> (1)..(57)

 <400> 114
 atg aaa cac ctg tgg ttc ttc ctc ctg ctg gtg gca gct ccc aga tgg 48
 Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
 1 5 10 15

 gtg ctg agc cag atc cag ctg gtg cag agc ggc gcc gag gtg aag aag 96
 Val Leu Ser Gln Ile Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
 20 25 30

 ccc ggc agc agc gtg aag gtg agc tgc aag gcc agc ggc tac acc ttc 144
 Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45

 acc cac tac agc atg cac tgg gtg cgg cag gcc ccc ggc cag ggc ctg 192
 Thr His Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
 50 55 60

 aag tgg atg ggc tgg atc aac acc tac acc ggc gag ccc acc tac gcc 240
 Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala
 65 70 75 80

 gac gac ttc aag ggc cgg ttc gcc ttc agc ctg gac acc tcc acc tcc 288
 Asp Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Thr Ser
 85 90 95

 acc gcc tac ctg gaa ctg agc agc ctg cgg agc gag gac acc gcc gtg 336
 Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
 100 105 110

tac tac tgc gcc acc tac tac cgg tac gag cgg gac ttc gac tac tgg	384
Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp	
115 120 125	
ggc cag ggc acc ctg gtg acc gtg agc tca gcc tcc acc aag ggc cca	432
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro	
130 135 140	
agc gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggc ggc aca	480
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr	
145 150 155 160	
gcc gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa ccc gtg acc	528
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr	
165 170 175	
gtg agc tgg aac tca ggc gcc ctg acc agc ggc gtg cac acc ttc ccc	576
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro	
180 185 190	
gct gtc ctg cag tcc tca gga ctc tac tcc ctc agc agc gtg gtg acc	624
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr	
195 200 205	
gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac gtg aat	672
Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn	
210 215 220	
cac aag ccc agc aac acc aag gtg gac aag aga gtt gag ccc aaa tct	720
His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser	
225 230 235 240	
tgt gac aaa act cac aca tgc cca ccc tgc cca gca cct gaa ctc ctg	768
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu	
245 250 255	
ggg gga ccc tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc	816
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu	
260 265 270	
atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc	864
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser	
275 280 285	
cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag	912
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu	
290 295 300	
gtg cat aat gcc aag aca aag ccc cgg gag gag cag tac aac agc acg	960
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr	
305 310 315 320	
tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat	1008
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn	
325 330 335	
ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc	1056
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro	
340 345 350	
atc gag aaa acc atc tcc aaa gcc aaa ggc cag ccc cgg gaa cca cag	1104
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln	
355 360 365	
gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc	1152
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val	
370 375 380	

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg	1200
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val	
385 390 395 400	
gag tgg gag agc aat ggc cag ccc gag aac aac tac aag acc acc cct	1248
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro	
405 410 415	
ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc	1296
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr	
420 425 430	
gtg gac aag agc agg tgg cag cag ggc aac gtc ttc tca tgc tcc gtg	1344
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val	
435 440 445	
atg cat gag gct ctg cac aac cac tac acc cag aag agc ctc tcc ctg	1392
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu	
450 455 460	
tct ccc ggc aaa tga	1407
Ser Pro Gly Lys	
465	

<210> 115
 <211> 468
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 115

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
 1 5 10 15

Val Leu Ser Gln Ile Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
 20 25 30

Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45

Thr His Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
 50 55 60

Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala
 65 70 75 80

Asp Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Thr Ser
 85 90 95

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

Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp
 115 120 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
130 135 140

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
145 150 155 160

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
165 170 175

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
180 185 190

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
195 200 205

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
210 215 220

His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
225 230 235 240

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
245 250 255

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
260 265 270

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
275 280 285

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
290 295 300

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
305 310 315 320

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
325 330 335

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
340 345 350

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
355 360 365

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
370 375 380

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
385 390 395 400

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
405 410 415

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
420 425 430

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
435 440 445

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
450 455 460

Ser Pro Gly Lys
465

<210> 116
<211> 15
<212> DNA
<213> Artificial

<220>
<223> hSH348-1-T3H CDRH1

<220>
<221> CDS
<222> (1)..(15)

<400> 116
cac tac agc atg cac
His Tyr Ser Met His
1 5

15

<210> 117
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 117

His Tyr Ser Met His
1 5

<210> 118
<211> 51
<212> DNA
<213> Artificial

<220>
<223> hSH348-1-T3H CDRH2

<220>
<221> CDS
<222> (1)..(51)

<400> 118
tgg atc aac acc tac acc ggc gag ccc acc tac gcc gac gac ttc aag

48

Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
 1 5 10 15

ggc
 Gly

51

<210> 119
 <211> 17
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 119

Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
 1 5 10 15

Gly

<210> 120
 <211> 30
 <212> DNA
 <213> Artificial

<220>
 <223> hSH348-1-T3H CDRH3

<220>
 <221> CDS
 <222> (1)..(30)

<400> 120
 tac tac cgg tac gag cgg gac ttc gac tac
 Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

30

<210> 121
 <211> 10
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 121

Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

<210> 122
 <211> 720
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T1L

<220>
 <221> CDS
 <222> (1)..(720)

<220>
 <221> sig_peptide
 <222> (1)..(60)

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<400> 122
atg gtg ctg cag acc cag gtg ttc atc tcc ctg ctg ctg tgg atc tcc      48
Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser
1                               5                               10          15

ggc gca tat ggc gac ata gtg atg acc cag agc ccc ctg agc ctg ccc      96
Gly Ala Tyr Gly Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro
20                               25                               30

gtg acc ccc ggc gag ccc gcc agc atc agc tgc cgg agc agc cag agc      144
Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
35                               40                               45

atc gtg cac agc agc ggc atc acc tac ctg gag tgg tac ctg cag aag      192
Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu Trp Tyr Leu Gln Lys
50                               55                               60

ccc ggc cag agc ccc cag ctg ctg atc tac aag gtg agc aac cgg ttc      240
Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe
65                               70                               75          80

agc ggc gtg ccc gac cgg ttc agc ggc agc ggc agc ggc acc gac ttc      288
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
85                               90                               95

acc ctg aag atc agc cgg gtg gag gcc gag gac gtg ggc gtg tac tac      336
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
100                              105                              110

tgc ttc cag ggc agc cac gtg ccc tac acc ttc ggc cag ggc acc aag      384
Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys
115                              120                              125

gtg gag atc aag cgt acg gtg gcc gcc ccc tcc gtg ttc atc ttc ccc      432
Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130                              135                              140

ccc tcc gac gag cag ctg aag tcc ggc acc gcc tcc gtg gtg tgc ctg      480
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145                              150                              155          160

ctg aat aac ttc tac ccc aga gag gcc aag gtg cag tgg aag gtg gac      528
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165                              170                              175

aac gcc ctg cag tcc ggg aac tcc cag gag agc gtg acc gag cag gac      576
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180                              185                              190

agc aag gac agc acc tac agc ctg agc agc acc ctg acc ctg agc aaa      624
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
195                              200                              205

gcc gac tac gag aag cac aag gtg tac gcc tgc gag gtg acc cac cag      672
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
210                              215                              220

ggc ctg agc tcc ccc gtc acc aag agc ttc aac agg ggg gag tgt tag      720
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                              230                              235

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<210> 123
 <211> 239
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 123

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 Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro
 20 25 30

Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
 35 40 45

Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu Trp Tyr Leu Gln Lys
 50 55 60

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys 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 Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys
 115 120 125

Val Glu 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
 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> 124
 <211> 48
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T1L CDRL1

<220>
 <221> CDS
 <222> (1)..(48)

<400> 124
 cgg agc agc cag agc atc gtg cac agc agc ggc atc acc tac ctg gag 48
 Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu
 1 5 10 15

<210> 125
 <211> 16
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 125
 Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu
 1 5 10 15

<210> 126
 <211> 21
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T1L CDRL2

<220>
 <221> CDS
 <222> (1)..(21)

<400> 126
 aag gtg agc aac cgg ttc agc 21
 Lys Val Ser Asn Arg Phe Ser
 1 5

<210> 127
 <211> 7
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 127
 Lys Val Ser Asn Arg Phe Ser
 1 5

<210> 128
 <211> 24
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T1L_CDRL3

<220>
 <221> CDS
 <222> (1)..(24)

<400> 128
 ttc cag ggc agc cac gtg ccc tac
 Phe Gln Gly Ser His Val Pro Tyr
 1 5

24

<210> 129
 <211> 8
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 129
 Phe Gln Gly Ser His Val Pro Tyr
 1 5

<210> 130
 <211> 720
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T3L

<220>
 <221> CDS
 <222> (1)..(720)

<220>
 <221> sig_peptide
 <222> (1)..(60)

<400> 130
 atg gtg ctg cag acc cag gtg ttc atc tcc ctg ctg ctg tgg atc tcc
 Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser
 1 5 10 15

48

ggc gca tat ggc gac gtg ctg atg acc cag agc ccc ctg agc ctg ccc
 Gly Ala Tyr Gly Asp Val Leu Met Thr Gln Ser Pro Leu Ser Leu Pro
 20 25 30

96

gtg acc ccc ggc gag ccc gcc agc atc agc tgc cgg agc agc cag agc
 Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
 35 40 45

144

atc gtg cac agc agc ggc atc acc tac ctg gag tgg tac ctg cag aag
 Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu Trp Tyr Leu Gln Lys
 50 55 60

192

ccc ggc cag agc ccc cag ctg ctg atc tac aag gtg agc aac cgg ttc Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe 65 70 75 80	240
agc ggc gtg ccc gac cgg ttc agc ggc agc ggc agc ggc acc gac ttc Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe 85 90 95	288
acc ctg aag atc agc cgg gtg gag gcc gag gac gtg ggc gtg tac tac Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr 100 105 110	336
tgc ttc cag ggc agc cac gtg ccc tac acc ttc ggc cag ggc acc aag Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys 115 120 125	384
gtg gag atc aag cgt acg gtg gcc gcc ccc tcc gtg ttc atc ttc ccc Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro 130 135 140	432
ccc tcc gac gag cag ctg aag tcc ggc acc gcc tcc gtg gtg tgc ctg Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu 145 150 155 160	480
ctg aat aac ttc tac ccc aga gag gcc aag gtg cag tgg aag gtg gac Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp 165 170 175	528
aac gcc ctg cag tcc ggg aac tcc cag gag agc gtg acc gag cag gac Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp 180 185 190	576
agc aag gac agc acc tac agc ctg agc agc acc ctg acc ctg agc aaa Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys 195 200 205	624
gcc gac tac gag aag cac aag gtg tac gcc tgc gag gtg acc cac cag Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln 210 215 220	672
ggc ctg agc tcc ccc gtc acc aag agc ttc aac agg ggg gag tgt tag Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys 225 230 235	720

<210> 131
 <211> 239
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 131

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 Val Leu Met Thr Gln Ser Pro Leu Ser Leu Pro
 20 25 30

Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
 35 40 45

Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu Trp Tyr Leu Gln Lys
 50 55 60
 Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Lys 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 Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gln Gly Thr Lys
 115 120 125
 Val Glu 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
 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> 132
 <211> 48
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T3L CDRL1

<220>
 <221> CDS
 <222> (1)..(48)

<400> 132
 cgg agc agc cag agc atc gtg cac agc agc ggc atc acc tac ctg gag
 Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu
 1 5 10 15

48

<210> 133

<211> 16
 <212> PRT
 <213> Artificial

 <220>
 <223> Synthetic Construct

 <400> 133
 Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Ile Thr Tyr Leu Glu
 1 5 10 15

<210> 134
 <211> 21
 <212> DNA
 <213> Artificial

 <220>
 <223> hSH357-1-T3L CDRL2

<220>
 <221> CDS
 <222> (1)..(21)

<400> 134
 aag gtg agc aac cgg ttc agc
 Lys Val Ser Asn Arg Phe Ser
 1 5

21

<210> 135
 <211> 7
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 135

Lys Val Ser Asn Arg Phe Ser
1 5

<210> 136
 <211> 24
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T3L CDRL3

<220>
 <221> CDS
 <222> (1)..(24)

<400> 136
 ttc cag ggc agc cac gtg ccc tac
 Phe Gln Gly Ser His Val Pro Tyr
 1 5

24

<210> 137
 <211> 8
 <212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 137

Phe Gln Gly Ser His Val Pro Tyr
1 5

<210> 138

<211> 1407

<212> DNA

<213> Artificial

<220>

<223> hsh357-1-T1H

<220>

<221> CDS

<222> (1)..(1407)

<220>

<221> sig_peptide

<222> (1)..(57)

<400> 138

atg aaa cac ctg tgg ttc ttc ctc ctg ctg gtg gca gct ccc aga tgg	48
Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp	
1 5 10 15	
gtg ctg agc cag gtg cag ctg gtg cag tcc ggc gcc gag gtg aag aag	96
Val Leu Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys	
20 25 30	
ccc ggc gcc tcc gtg aag gtg tcc tgt aag gcc tcc ggc tac acc ttc	144
Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe	
35 40 45	
atc gac tac tcc atg cac tgg gtg agg cag gcc ccc ggc cag ggc ctg	192
Ile Asp Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu	
50 55 60	
gag tgg atg ggc tgg atc aac acc tac acc ggc gag ccc acc tac tcc	240
Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser	
65 70 75 80	
gac gac ttc aag ggc agg gtg acc atc acc gcc gac acc tcc acc agc	288
Asp Asp Phe Lys Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser	
85 90 95	
acc gcc tac ctg gag ctg tcc tcc ctg agg tcc gag gac acc gcc gtg	336
Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val	
100 105 110	
tac tac tgt gcc acc tac tac agg tac gag agg gac ttc gac tac tgg	384
Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp	
115 120 125	
ggc cag ggc acc ctg gtg acg gtg agc tca gcc tcc acc aag ggc cca	432
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro	
130 135 140	
agc gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggc ggc aca	480
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr	
145 150 155 160	

gcc gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa ccc gtg acc	528
Ala Ala Leu Gly Cys 165 Leu Val Lys Asp Tyr 170 Phe Pro Glu Pro Val Thr 175	
gtg agc tgg aac tca ggc gcc ctg acc agc ggc gtg cac acc ttc ccc	576
Val Ser Trp Asn 180 Ser Gly Ala Leu Thr 185 Ser Gly Val His Thr 190 Phe Pro	
gct gtc ctg cag tcc tca gga ctc tac tcc ctc agc agc gtg gtg acc	624
Ala Val Leu 195 Gln Ser Ser Gly 200 Leu Tyr Ser Leu Ser Ser Val Val Thr	
gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac gtg aat	672
Val Pro 210 Ser Ser Ser Leu Gly 215 Thr Gln Thr Tyr Ile 220 Cys Asn Val Asn	
cac aag ccc agc aac acc aag gtg gac aag aga gtt gag ccc aaa tct	720
His Lys Pro Ser Asn 230 Thr Lys Val Asp Lys Arg 235 Val Glu Pro Lys Ser 240	
tgt gac aaa act cac aca tgc cca ccc tgc cca gca cct gaa ctc ctg	768
Cys Asp Lys Thr 245 His Thr Cys Pro Pro Cys 250 Pro Ala Pro Glu Leu Leu 255	
ggg gga ccc tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc	816
Gly Gly Pro Ser 260 Val Phe Leu Phe 265 Pro Pro Lys Pro Lys Asp Thr Leu	
atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc	864
Met Ile Ser 275 Arg Thr Pro Glu Val 280 Thr Cys Val Val 285 Asp Val Ser	
cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag	912
His Glu 290 Asp Pro Glu Val 295 Lys Phe Asn Trp Tyr 300 Val Asp Gly Val Glu	
gtg cat aat gcc aag aca aag ccc cgg gag gag gag cag tac aac agc acg	960
Val His Asn Ala Lys 310 Thr Lys Pro Arg Glu Glu 315 Gln Tyr Asn Ser Thr 320	
tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat	1008
Tyr Arg Val Val 325 Ser Val Leu Thr Val 330 His Gln Asp Trp Leu Asn 335	
ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc	1056
Gly Lys Glu Tyr 340 Lys Cys Lys Val Ser 345 Asn Lys Ala Leu Pro Ala Pro 350	
atc gag aaa acc atc tcc aaa gcc aaa ggc cag ccc cgg gaa cca cag	1104
Ile Glu Lys 355 Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln 365	
gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc	1152
Val Tyr Thr Leu Pro Pro 375 Ser Arg Glu Glu Met Thr 380 Lys Asn Gln Val	
agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg	1200
Ser Leu Thr Cys Leu 390 Val Lys Gly Phe Tyr Pro 395 Ser Asp Ile Ala Val 400	
gag tgg gag agc aat ggc cag ccc gag aac aac tac aag acc acc cct	1248
Glu Trp Glu Ser Asn 405 Gly Gln Pro Glu Asn 410 Asn Tyr Lys Thr Thr Pro 415	
ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc	1296
Pro Val Leu 420 Asp Ser Asp Gly Ser Phe 425 Phe Leu Tyr Ser Lys 430 Leu Thr	

gtg gac aag agc agg tgg cag cag ggc aac gtc ttc tca tgc tcc gtg 1344
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
435 440 445

atg cat gag gct ctg cac aac cac tac acc cag aag agc ctc tcc ctg 1392
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
450 455 460

tct ccc ggc aaa tga 1407
Ser Pro Gly Lys
465

<210> 139
<211> 468
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 139

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1 5 10 15

Val Leu Ser Gln val Gln Leu val Gln Ser Gly Ala Glu val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Ile Asp Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser
65 70 75 80

Asp Asp Phe Lys Gly Arg val Thr Ile Thr Ala Asp Thr Ser Thr Ser
85 90 95

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

Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
130 135 140

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
145 150 155 160

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
165 170 175

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
180 185 190

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
195 200 205

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
210 215 220

His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
225 230 235 240

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
245 250 255

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
260 265 270

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
275 280 285

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
290 295 300

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
305 310 315 320

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
325 330 335

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
340 345 350

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
355 360 365

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
370 375 380

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
385 390 395 400

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
405 410 415

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
420 425 430

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
435 440 445

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
 450 455 460

Ser Pro Gly Lys
 465

<210> 140
 <211> 18
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T1H CDRH1

<220>
 <221> CDS
 <222> (1)..(18)

<400> 140
 atc gac tac tcc atg cac
 Ile Asp Tyr Ser Met His
 1 5

18

<210> 141
 <211> 6
 <212> PRT
 <213> Artificial

<220>
 <223> synthetic Construct

<400> 141

Ile Asp Tyr Ser Met His
 1 5

<210> 142
 <211> 51
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T1H CDRH2

<220>
 <221> CDS
 <222> (1)..(51)

<400> 142
 tgg atc aac acc tac acc ggc gag ccc acc tac tcc gac gac ttc aag
 Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe Lys
 1 5 10 15

48

ggc
 Gly

51

<210> 143
 <211> 17
 <212> PRT
 <213> Artificial

<220>
<223> Synthetic Construct

<400> 143

Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe Lys
1 5 10 15

Gly

<210> 144
<211> 30
<212> DNA
<213> Artificial

<220>
<223> hSH357-1-T1H CDRH3

<220>
<221> CDS
<222> (1)..(30)

<400> 144
tac tac agg tac gag agg gac ttc gac tac
Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
1 5 10

30

<210> 145
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 145

Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
1 5 10

<210> 146
<211> 1407
<212> DNA
<213> Artificial

<220>
<223> hSH357-1-T3H

<220>
<221> CDS
<222> (1)..(1407)

<220>
<221> sig_peptide
<222> (1)..(57)

<400> 146
atg aaa cac ctg tgg ttc ttc ctc ctg ctg gtg gca gct ccc aga tgg
Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1 5 10 15

48

gtg ctg agc cag atc cag ctg gtg cag tcc gcc gcc gag gtg aag aag Val Leu Ser Gln Ile Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys	96
ccc gcc gcc tcc gtg aag gtg tcc tgt aag gcc tcc gcc tac acc ttc Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe	144
atc gac tac tcc atg cac tgg gtg agg cag gcc ccc gcc cag gcc ctg Ile Asp Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu	192
aag tgg atg gcc tgg atc aac acc tac acc gcc gag ccc acc tac tcc Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser	240
gac gac ttc aag gcc agg ttc gcc ttc tcc ctg gac acc tcc acc agc Asp Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Thr Ser	288
acc gcc tac ctg gag ctg tcc tcc ctg agg tcc gag gac acc gcc gtg Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val	336
tac tac tgt gcc acc tac tac agg tac gag agg gac ttc gac tac tgg Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp	384
gcc cag gcc acc ctg gtg acg gtg agc tca gcc tcc acc aag gcc cca Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro	432
agc gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct gcc gcc aca Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr	480
gcc gcc ctg gcc tgc ctg gtc aag gac tac ttc ccc gaa ccc gtg acc Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr	528
gtg agc tgg aac tca gcc gcc ctg acc agc gcc gtg cac acc ttc ccc Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro	576
gct gtc ctg cag tcc tca gga ctc tac tcc ctc agc agc gtg gtg acc Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr	624
gtg ccc tcc agc agc ttg gcc acc cag acc tac atc tgc aac gtg aat Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn	672
cac aag ccc agc aac acc aag gtg gac aag aga gtt gag ccc aaa tct His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser	720
tgt gac aaa act cac aca tgc cca ccc tgc cca gca cct gaa ctc ctg Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu	768
ggg gga ccc tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu	816
atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser	864

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu 290 295 300	912
gtg cat aat gcc aag aca aag ccc cgg gag gag cag tac aac agc acg Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr 305 310 315 320	960
tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn 325 330 335	1008
ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro 340 345 350	1056
atc gag aaa acc atc tcc aaa gcc aaa ggc cag ccc cgg gaa cca cag Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln 355 360 365	1104
gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val 370 375 380	1152
agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val 385 390 395 400	1200
gag tgg gag agc aat ggc cag ccc gag aac aac tac aag acc acc cct Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro 405 410 415	1248
ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr 420 425 430	1296
gtg gac aag agc agg tgg cag cag ggc aac gtc ttc tca tgc tcc gtg Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val 435 440 445	1344
atg cat gag gct ctg cac aac cac tac acc cag aag agc ctc tcc ctg Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu 450 455 460	1392
tct ccc ggc aaa tga Ser Pro Gly Lys 465	1407

<210> 147
 <211> 468
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 147

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1 5 10 15

Val Leu Ser Gln Ile Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45
 Ile Asp Tyr Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
 50 55 60
 Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser
 65 70 75 80
 Asp Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Thr Ser
 85 90 95
 Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
 100 105 110
 Tyr Tyr Cys Ala Thr Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr Trp
 115 120 125
 Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 130 135 140
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 145 150 155 160
 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 165 170 175
 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 180 185 190
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 195 200 205
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 210 215 220
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
 225 230 235 240
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 245 250 255
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 260 265 270
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 275 280 285
 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 290 295 300

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 305 310 315 320
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 325 330 335
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 340 345 350
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 355 360 365
 Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
 370 375 380
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 385 390 395 400
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 405 410 415
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 420 425 430
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
 435 440 445
 Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
 450 455 460

Ser Pro Gly Lys
465

<210> 148
 <211> 18
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T3H CDRH1

<220>
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 <222> (1)..(18)

<400> 148
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 Ile Asp Tyr Ser Met His
 1 5

18

<210> 149
 <211> 6
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct
 <400> 149

Ile Asp Tyr Ser Met His
 1 5

<210> 150
 <211> 51
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T3H CDRH2

<220>
 <221> CDS
 <222> (1)..(51)

<400> 150
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 Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ser Asp Asp Phe Lys
 1 5 10 15

ggc 51
 Gly

<210> 151
 <211> 17
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 151
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 1 5 10 15

Gly

<210> 152
 <211> 30
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T3H CDRH3

<220>
 <221> CDS
 <222> (1)..(30)

<400> 152
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 Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

<210> 153
 <211> 10
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 153

Tyr Tyr Arg Tyr Glu Arg Asp Phe Asp Tyr
 1 5 10

<210> 154
 <211> 520
 <212> DNA
 <213> Artificial

<220>
 <223> IgGL constant+signal

<400> 154
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 tgcagaccca ggtgttcac tccctgctgc tgtggatctc cggcgcatat ggcgatatcg 120
 tgatgattaa acgtacgggt gccgccccct ccgtgttcat cttccccccc tccgacgagc 180
 agctgaagtc cggcaccgcc tccgtggtgt gcctgctgaa taacttctac cccagagagg 240
 ccaagggtgca gtggaagggt gacaacgccc tgcagtccgg gaactcccag gagagcgtga 300
 ccgagcagga cagcaaggac agcacctaca gcctgagcag caccctgacc ctgagcaaa 360
 ccgactacga gaagcacaag gtgtacgcct gcgagggtgac ccaccagggc ctgagctccc 420
 ccgtcaccaa gagcttcaac aggggggaggt gttaggggcc cgtttaaacg ggtggcatcc 480
 ctgtgacccc tccccagtgc ctctcctggc cctggaagtt 520

<210> 155
 <211> 1140
 <212> DNA
 <213> Artificial

<220>
 <223> IgGH constant+signal

<400> 155
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 gttagctcag cctccaccaa gggcccaagc gtcttcccc tggcaccctc ctccaagagc 180
 acctctggcg gcacagccgc cctgggctgc ctggtcaagg actacttccc cgaaccctgt 240
 accgtgagct ggaactcagg cgccttgacc agcggcgtgc acaccttccc cgctgtcctg 300
 cagtcctcag gactctactc cctcagcagc gtgggtgaccg tgccctccag cagcttgggc 360
 acccagacct acatctgcaa cgtgaatcac aagcccagca acaccaaggt ggacaagaga 420
 gttgagccca aatcttgtga caaaactcac acatgccac cctgcccagc acctgaactc 480

ctgggggggac cctcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc	540
cggacccctg aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag	600
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cagtacaaca gcacgtaccg ggtggtcagc gtcctcaccg tcctgcacca ggactggctg	720
aatggcaagg agtacaagtg caaggctctc aacaaagccc tcccagcccc catcgagaaa	780
accatctcca aagccaaagg ccagccccgg gaaccacagg tgtacaccct gccccatcc	840
cgggaggaga tgaccaagaa ccaggtcagc ctgacctgcc tggtaaagg cttctatccc	900
agcgacatcg ccgtggagtg ggagagcaat ggccagcccc agaacaacta caagaccacc	960
cctcccgtgc tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag	1020
agcaggtggc agcagggcaa cgtcttctca tgctccgtga tgcattaggc tctgcacaac	1080
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<210> 156
 <211> 442
 <212> DNA
 <213> Artificial

<220>
 <223> hSH348-1-T1L+signal

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gacccccggc gagccgcct ccatcagctg ccggagcagc cagagcatcg tgcacagcaa	180
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caccgacttc accctgaaga tcagccgggt ggaggccgag gacgtgggcg tgtactactg	360
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<210> 157
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 <212> DNA
 <213> Artificial

<220>
 <223> hSH348-1-T3L+signal

<400> 157	
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gacccccggc gagccgcct ccatcagctg ccggagcagc cagagcatcg tgcacagcaa	180
cggcaacacc tacctggagt ggtacctgca gaagcccggc cagagcccc agctgctgat	240
ctacaagggt agcaaccggt tcagcggcgt gcccgaccgg ttcagcggca gcggcagcgg	300

caccgacttc accctgaaga tcagccgggt ggaggccgag gacgtgggcg tgtactactg	360
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<210> 158
 <211> 460
 <212> DNA
 <213> Artificial

<220>
 <223> hSH348-1-T1H+signal

<400> 158	
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cagcagcgtg aagggtgagct gcaaggccag cggctacacc ttcacccact acagcatgca	180
ctgggtgcgg caggcccccg gccagggcct ggagtggatg ggctggatca acacctacac	240
cggcgagccc acctacgccg acgacttcaa gggccgggtg accatcaccg ccgacacctc	300
cacctccacc gcctacctgg aactgagcag cctgcggagc gaggacaccg ccgtgtacta	360
ctgcgccacc tactaccggt acgagcggga cttcgactac tggggccagc gcaccctggt	420
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<210> 159
 <211> 460
 <212> DNA
 <213> Artificial

<220>
 <223> hSH348-1-T3H+signal

<400> 159	
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cagcagcgtg aagggtgagct gcaaggccag cggctacacc ttcacccact acagcatgca	180
ctgggtgcgg caggcccccg gccagggcct gaagtggatg ggctggatca acacctacac	240
cggcgagccc acctacgccg acgacttcaa gggccgggtc gccttcagcc tggacacctc	300
cacctccacc gcctacctgg aactgagcag cctgcggagc gaggacaccg ccgtgtacta	360
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<210> 160
 <211> 442
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T1L+signal

<400> 160

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gacccccggc gagcccccca gcatcagctg ccggagcagc cagagcatcg tgcacagcag	180
cggcatcacc tacctggagt ggtacctgca gaagcccggc cagagcccc agctgctgat	240
ctacaagggtg agcaaccggt tcagcggcgt gcccgaccgg ttcagcggca gcggcagcgg	300
caccgacttc accctgaaga tcagccgggt ggaggccgag gacgtgggcg tgtactactg	360
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 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T3L+signal

<400> 161	
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gacccccggc gagcccccca gcatcagctg ccggagcagc cagagcatcg tgcacagcag	180
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caccgacttc accctgaaga tcagccgggt ggaggccgag gacgtgggcg tgtactactg	360
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tacggtggcc gccccctccg tg	442

<210> 162
 <211> 460
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T1H+signal

<400> 162	
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cgcctccgtg aaggtgtcct gtaaggcctc cggctacacc ttcatcgact actccatgca	180
ctgggtgagg caggcccccg gccagggcct ggagtggatg ggctggatca acacctacac	240
cggcgagccc acctactccg acgacttcaa gggcagggtg accatcaccg ccgacacctc	300
caccagcacc gcctacctgg agctgtcctc cctgaggtcc gaggacaccg ccgtgtracta	360
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<210> 163
 <211> 460
 <212> DNA
 <213> Artificial

<220>
 <223> hSH357-1-T3H+signal

<400> 163
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 cgcctccgtg aaggtgtcct gtaaggcctc cggctacacc ttcacgact actccatgca 180
 ctgggtgagg caggcccccg gccagggcct gaagtggatg ggctggatca acacctacac 240
 cggcgagccc acctactccg acgacttcaa gggcagggtc gccttctccc tggacacctc 300
 caccagcacc gcctacctgg agctgtcctc cctgaggtcc gaggacaccg ccgtgtacta 360
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