



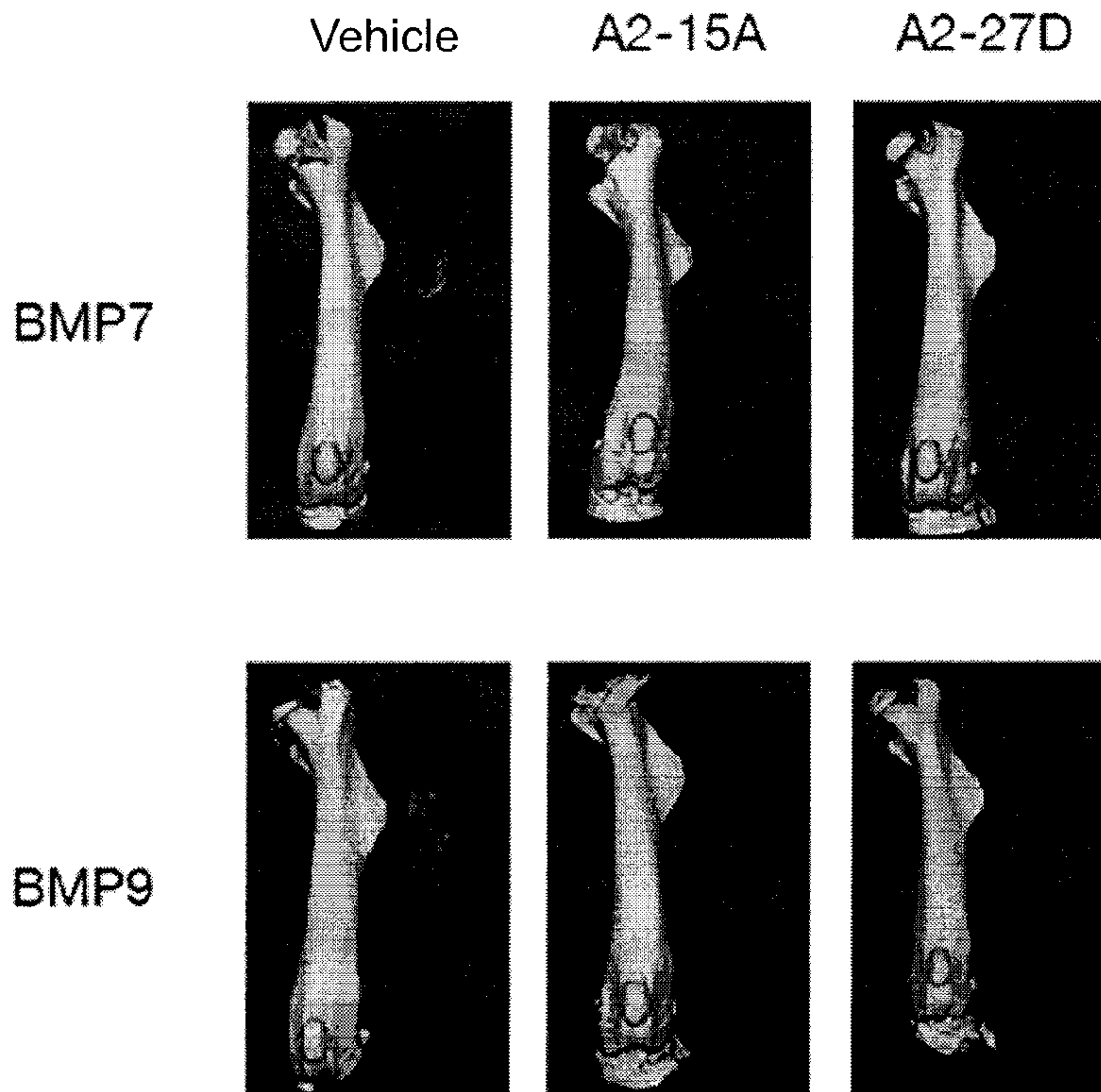
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(54) Titre : ANTICORPS ANTI-ALK2
(54) Title: ANTI-ALK2 ANTIBODY



(57) **Abrégé/Abstract:**

Provided are: an antibody which specifically binds to an ALK2 protein and has an activity of inhibiting BMP signal transduction mediated by ALK2; a method for producing the antibody; and a pharmaceutical composition containing the antibody for treating and/or preventing heterotopic ossification and/or osteodystrophy, anemia, or diffuse intrinsic pontine glioma (DIPG).

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ABSTRACT

This application provides: an antibody which specifically binds to an ALK2 protein and has an activity of inhibiting BMP signal transduction mediated by ALK2; a method for producing the antibody; and a pharmaceutical composition comprising the antibody, for treating and/or preventing ectopic ossification and/or bone dysplasia, anemia, or diffuse intrinsic pontine glioma (DIPG).

DESCRIPTION

ANTI-ALK2 ANTIBODY

TECHNICAL FIELD

[0001]

The present invention relates to a substance useful as a therapeutic and/or prophylactic drug for ectopic ossification and/or bone dysplasia, and to a method for treating and/or preventing ectopic ossification and/or bone dysplasia.

BACKGROUND ART

[0002]

Fibrodysplasia ossificans progressiva (FOP) is a genetic disease which causes cartilage tissues or bone tissues to be ectopically formed in soft tissues, such as skeletal muscle, tendon, and ligament, where bone tissues are not normally formed (Non Patent Literatures 1 to 3). In this disease, ectopic ossification occurs throughout the entire body including the face so that ectopic bones and existing normal bones are fused to remarkably reduce the range of joint motion or to deform the body (Non Patent Literatures 1 to 3).

[0003]

The ectopic ossification in FOP proceeds chronically with growth, whereas acute ectopic ossification is also known which proceeds while manifesting a symptom called flare-up caused by muscle injury, viral infection, or the like (Non Patent Literature 1). The flare-up is swelling with inflammatory response or sustained pain as principal symptoms. The flare-up is induced by bruise, falling, intramuscular injection, or the like, which causes muscle injury. In addition, sudden flare-ups with no clear cause are also known. For FOP, invasive medical acts such as biopsy and operation are contraindicated because ectopic bones are formed after flare-up. Thus, the ectopic bone tissues cannot be surgically removed. The ectopic bone tissues in FOP are consisted of normal cartilage cells or osteoblasts and bone

metabolism of heterotopic bones is the same as normal bone tissues. As such, only the ectopic bone tissues cannot be medically removed using drugs or the like.

[0004]

Any fundamental therapy for suppressing the ectopic ossification in FOP has not yet been established, and only symptomatic treatment for pain or the like has been made. Thus, the ectopic bone tissues formed in FOP are very difficult to remove, and the development of a promising drug that can exert prophylactic effects before the onset of ectopic ossification has been expected.

[0005]

Activin like kinase 2 (ALK2) gene encoding a receptor of bone morphogenetic proteins (BMPs) that induces ectopic bone formation in soft tissues including skeletal muscle tissues has been identified as a responsible gene for FOP (Non Patent Literature 4). The ALK2 gene is identical to activin A type I receptor 1 (ACVR1) gene. ALK2 having an amino acid substitution has been found from familial and sporadic FOP cases (Non Patent Literature 4).

[0006]

Human or mouse ALK2 is a single-pass transmembrane protein consisting of 509 amino acids and having a signal peptide and functions as a transmembrane type of serine-threonine kinase receptor binding to BMPs (Non Patent Literatures 1 to 3). ALK2 binds to BMPs at its N-terminal extracellular region and activates the downstream intracellular signal transduction system through an intracellular serine-threonine kinase.

[0007]

BMP receptors are classified based on their structures and functions into 2 types: type I receptors including ALK2; and type II receptors (Non Patent Literatures 1 to 3). The type II receptors are constitutively active enzymes that exhibit kinase activity even if not bound with BMP. On the other hand, the type I receptors including ALK2 are inactive enzymes in a state unbound with BMP and exhibit kinase activity in a manner dependent on binding to BMP. This is probably because upon binding to BMP, type II receptor kinase phosphorylates type I receptor intracellular domain as the substrate, which can change its conformation, and activates the type I receptor (Non Patent Literatures 1 to 3).

[0008]

Type I receptors are known to be constitutive active independent of a type II receptor by substitution of a particular amino acid in the intracellular region (Non Patent Literatures 1 to 3). Overexpression of this constitutively active mutants of the type I receptors activate the intracellular signal transduction system without BMP stimulation. Thus, the type I receptors are considered as responsible molecules that transduce BMP signals from the outside to the inside of cells.

[0009]

A mutation in ALK2 identified from familial and typical sporadic FOP patients was the R206H mutation in which Arg206 is substituted by His (Non Patent Literature 4). All of gene mutations previously identified from FOP cases have been reported to cause amino acid substitutions in the intracellular region of ALK2. Most of these mutations from FOP cases reside mainly in or around the ATP-binding region in the intracellular domain of ALK2 (Non Patent Literature 5).

[0010]

Overexpression of the ALK2 mutants identified in FOP in cultured cells activates the intracellular signal transduction system of BMP without BMP stimulation (Non Patent Literature 6). Accordingly, for example, small molecular inhibitors for ALK2 kinase, RNAi or exon skipping methods which specifically suppress the expression of genetically mutated ALK2, downstream transcriptional factor inhibitors of ALK2 receptor, and inhibitors of BMP signal-mediated osteoblast differentiation are being developed as therapeutics for FOP (Patent Literature 1 and Non Patent Literatures 1 to 3).

[0011]

All of small molecular compounds or nucleic acids currently under development as therapeutics for FOP are expected to inhibit intracellular ALK2 signal after they penetrate cell membranes. However, any effective drug delivery method has not yet been established for nucleic acid drugs. In addition, there still remain problems such as the low specificity of kinase inhibitors for other ALK receptor families with high similarity to ALK2. As such, it has been desired to develop novel therapeutics for FOP having high specificity for ALK2.

Antibody drugs that act on the extracellular region of ALK2 and inhibit its signal are highly safe therapeutic approach that utilize the physiological immune system. The antibody drugs facilitate stable drug delivery via blood flow and are capable of specifically inhibiting ALK2 without acting on other ALK receptor families with high identity thereto. Furthermore, the antibody drugs can be expected to have inhibitory effect on wild-type ALK2 and various intracellular mutant ALK2s including novel unidentified mutants. The antibody drugs are expected not to inhibit ALK3 expressed in cells throughout the body. Therefore, unlike general osteoblast differentiation inhibitors, antibodies specifically inhibiting ALK2 can be expected to serve as drugs less likely to influence the growth, maintenance, and regeneration of normal skeletal tissues. However, any antibody that specifically binds to ALK2 and exhibits therapeutic effects on FOP has not been known so far.

PRIOR ART LITERATURE

Patent Literature

[0012]

Patent Literature 1: International Publication No. WO2007/123896

Non Patent Literature

[0013]

Non Patent Literature 1: T. Katagiri, J. Oral Biosci., 52, 33-41 (2010)

Non Patent Literature 2: T. Katagiri, J. Oral Biosci., 54, 119-123 (2012)

Non Patent Literature 3: T. Katagiri and S. Tsukamoto, Biol. Chem., 394, 703-714 (2013)

Non Patent Literature 4: E.M. Shore et al., Nat. Genet., 38, 525-527 (2006)

Non Patent Literature 5: A. Chaikuad et al., J. Biol. Chem., 287, 36990-36998 (2012)

Non Patent Literature 6: T. Fukuda et al., J. Biol. Chem., 284, 7149-7156 (2009)

SUMMARY OF INVENTION

Problem to be Solved by the Invention

[0014]

An object of the present invention is to provide a substance useful as a therapeutic and/or prophylactic drug for ectopic ossification and/or bone dysplasia, and a method for treating and/or preventing ectopic ossification and/or bone dysplasia.

Means for Solution of the Problem

[0015]

The present inventors have now conducted diligent studies to attain the object and consequently completed the present invention through successfully obtaining a novel antibody that specifically binds to ALK2 and has therapeutic and/or prophylactic effects on ectopic ossification and/or bone dysplasia.

[0016]

Specifically, the present invention encompasses the following aspects:

[0017]

(1) An antibody or an antigen-binding fragment thereof which specifically binds to a polypeptide sequence consisting of at least 7 amino acids in any one of the following amino acid sequences (a) to (e) and inhibits ALK2-mediated BMP signal transduction:

- (a) the amino acid sequence of SEQ ID NO: 84;
- (b) an amino acid sequence consisting of amino acid numbers 21 to 123 of the amino acid sequence of SEQ ID NO: 84;
- (c) the amino acid sequence of SEQ ID NO: 86;
- (d) an amino acid sequence consisting of amino acid numbers 21 to 123 of the amino acid sequence of SEQ ID NO: 86; and
- (e) an amino acid sequence comprising a substitution(s), deletion(s), or addition(s) of one to several amino acid residues in any one of the amino acid sequences (a) to (d).

[0018]

(2) An antibody or an antigen-binding fragment thereof which specifically binds to a polypeptide sequence consisting of at least 7 amino acids in an amino acid sequence encoded by any one of the following nucleotide sequences (f) to (j) and inhibits ALK2-mediated BMP signal transduction:

- (f) the nucleotide sequence of SEQ ID NO: 85;
- (g) a nucleotide sequence consisting of nucleotide numbers 728 to 1036 of the nucleotide sequence of SEQ ID NO: 85;
- (h) the nucleotide sequence of SEQ ID NO: 87;
- (i) a nucleotide sequence consisting of nucleotide numbers 728 to 1036 of the nucleotide sequence of SEQ ID NO: 87; and
- (j) a nucleotide sequence of a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of the nucleotide sequence complementary to any one of the nucleotide sequences (f) to (i).

[0019]

(3) The antibody or the antigen-binding fragment thereof according to (1) or (2), wherein the polypeptide sequence is a sequence in an ALK2 extracellular region.

[0020]

(4) The antibody or the antigen-binding fragment thereof according to any one of (1) to (3), wherein the antibody binds to a wild-type ALK2 protein and a mutant ALK2 protein.

[0021]

(5) The antibody or the antigen-binding fragment thereof according to any one of (1) to (4), wherein the antibody is a monoclonal antibody or a polyclonal antibody.

[0022]

(6) The antibody or the antigen-binding fragment thereof according to any one of (1) to (5), wherein the antibody or the antigen-binding fragment is a chimeric antibody, a humanized antibody, a human antibody, a single-chain antibody, a bispecific antibody, or a multispecific antibody.

[0023]

(7) The antibody or the antigen-binding fragment thereof according to any one of (1) to (6), wherein the antibody or the antigen-binding fragment cross-competes, for binding to the polypeptide, with at least any one antibody selected from the group consisting of an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 2 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO:

4, an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 6 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 8, an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 10 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 12, and an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 14 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 16.

[0024]

(8) The antibody or the antigen-binding fragment thereof according to any one of (1) to (6), wherein the antibody or the antigen-binding fragment binds to an epitope that is bound by at least any one antibody selected from the group consisting of an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 2 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 4, an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 6 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 8, an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 10 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 12, and an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 14 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 16.

[0025]

(9) The antibody or the antigen-binding fragment thereof according to any one of (1) to (6), wherein the antibody or the antigen-binding fragment binds to an epitope comprising each residue of glutamic acid (Glu) at position 18, glycine (Gly) at position 19, isoleucine (Ile) at position 39, asparagine (Asn) at position 40, aspartic acid (Asp) at position 41, glycine (Gly) at position 42, phenylalanine (Phe) at position 43, histidine (His) at position 44, valine (Val) at position 45, tyrosine (Tyr) at position 46, asparagine (Asn) at position 82, threonine (Thr) at position 84, glutamine (Gln) at position 86, leucine (Leu) at position 87, proline (Pro) at position 88, and threonine (Thr) at position 89 in the amino acid sequence of SEQ ID NO: 84.

[0026]

(10) The antibody or the antigen-binding fragment thereof according to any one of (1) to (6), having an interaction distance between the antibody or the antigen-binding fragment and each residue of glutamic acid (Glu) at position 18, glycine (Gly) at position 19, isoleucine (Ile) at position 39, asparagine (Asn) at position 40, aspartic acid (Asp) at position 41, glycine (Gly) at position 42, phenylalanine (Phe) at position 43, histidine (His) at position 44, valine (Val) at position 45, tyrosine (Tyr) at position 46, asparagine (Asn) at position 82, threonine (Thr) at position 84, glutamine (Gln) at position 86, leucine (Leu) at position 87, proline (Pro) at position 88, and threonine (Thr) at position 89 in the amino acid sequence of SEQ ID NO: 84.

[0027]

(11) The antibody or the antigen-binding fragment thereof according to any one of (1) to (6), wherein the antibody or the antigen-binding fragment binds to an epitope comprising each residue of glutamic acid (Glu) at position 18, glycine (Gly) at position 19, leucine (Leu) at position 20, isoleucine (Ile) at position 39, asparagine (Asn) at position 40, aspartic acid (Asp) at position 41, glycine (Gly) at position 42, phenylalanine (Phe) at position 43, histidine (His) at position 44, valine (Val) at position 45, tyrosine (Tyr) at position 46, and threonine (Thr) at position 84 in the amino acid sequence of SEQ ID NO: 84.

[0028]

(12) The antibody or the antigen-binding fragment thereof according to any one of (1) to (6), having an interaction distance between the antibody or the antigen-binding fragment and each residue of glutamic acid (Glu) at position 18, glycine (Gly) at position 19, leucine (Leu) at position 20, isoleucine (Ile) at position 39, asparagine (Asn) at position 40, aspartic acid (Asp) at position 41, glycine (Gly) at position 42, phenylalanine (Phe) at position 43, histidine (His) at position 44, valine (Val) at position 45, tyrosine (Tyr) at position 46, and threonine (Thr) at position 84 in the amino acid sequence of SEQ ID NO: 84.

[0029]

(13) The antibody or the antigen-binding fragment thereof according to (10) or (12), wherein the interaction distance is 6 angstroms or smaller.

[0030]

(14) The antibody or the antigen-binding fragment thereof according to (10) or (12), wherein the interaction distance is 4 angstroms or smaller.

[0031]

(15) The antibody or the antigen-binding fragment thereof according to any one of (1) to (8), wherein

the heavy chain sequence comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1 consists of the amino acid sequence of SEQ ID NO: 72, the CDRH2 consists of the amino acid sequence of SEQ ID NO: 73, and the CDRH3 consists of the amino acid sequence of SEQ ID NO: 74, and

the light chain sequence comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1 consists of the amino acid sequence of SEQ ID NO: 75, the CDRL2 consists of the amino acid sequence of SEQ ID NO: 76, and the CDRL3 consists of the amino acid sequence of SEQ ID NO: 77.

[0032]

(16) The antibody or the antigen-binding fragment thereof according to (15), wherein the antibody comprises a heavy chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 2 and a light chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 4.

[0033]

(17) The antibody or the antigen-binding fragment thereof according to any one of (1) to (8), wherein

the heavy chain sequence comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1 consists of an amino acid sequence of SEQ ID NO: 59, the CDRH2 consists of an amino acid sequence of SEQ ID NO: 60, and the CDRH3 consists of an amino acid sequence of SEQ ID NO: 61, and

the light chain sequence comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1 consists of the amino acid sequence of SEQ ID NO: 62, the CDRL2

consists of the amino acid sequence of SEQ ID NO: 63 or SEQ ID NO: 71, and the CDRL3 consists of the amino acid sequence of SEQ ID NO: 64.

[0034]

(18) The antibody or the antigen-binding fragment thereof according to (17), wherein the antibody comprises a heavy chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 6 and a light chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 8 or the amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 36.

[0035]

(19) The antibody or the antigen-binding fragment of the antibody according to any one of (1) to (10), (13) and (14), wherein

a heavy chain sequence comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1 consists of the amino acid sequence of SEQ ID NO: 78, the CDRH2 consists of the amino acid sequence of SEQ ID NO: 79, and the CDRH3 consists of the amino acid sequence of SEQ ID NO: 80; and

a light chain sequence comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1 consists of the amino acid sequence of SEQ ID NO: 81, the CDRL2 consists of the amino acid sequence of SEQ ID NO: 82, and the CDRL3 consists of the amino acid sequence of SEQ ID NO: 83.

[0036]

(20) The antibody or the antigen-binding fragment thereof according to (19), wherein the antibody comprises a heavy chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 10 and a light chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 12.

[0037]

(21) The antibody or the antigen-binding fragment thereof according to any one of (1) to (8) and (11) to (14), wherein

a heavy chain sequence comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1 consists of the amino acid sequence of SEQ ID NO: 65, the CDRH2

consists of the amino acid sequence of SEQ ID NO: 66, and the CDRH3 consists of the amino acid sequence of SEQ ID NO: 67; and

a light chain sequence comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1 consists of the amino acid sequence of SEQ ID NO: 68, the CDRL2 consists of the amino acid sequence of SEQ ID NO: 69, and the CDRL3 consists of the amino acid sequence of SEQ ID NO: 70.

[0038]

(22) The antibody or the antigen-binding fragment thereof according to (21), wherein the antibody comprises a heavy chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 14 and a light chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 16.

[0039]

(23) The antigen-binding fragment thereof according to any one of (1) to (22), wherein the antigen-binding fragment is selected from the group consisting of Fab, F(ab')₂, Fab', and Fv.

[0040]

(24) The antibody according to any one of (1) to (22), wherein the antibody is scFv.

[0041]

(25) The antibody or the antigen-binding fragment thereof according to any one of (1) to (22), wherein the antibody is a chimeric antibody.

[0042]

(26) The antibody or the antigen-binding fragment thereof according to any one of (1) to (22), wherein the antibody is humanized.

[0043]

(27) The antibody according to any one of (1) to (24), wherein the heavy chain comprises a constant region of a human immunoglobulin G1 heavy chain, a human immunoglobulin G2 heavy chain, or a human immunoglobulin G4 heavy chain, and the light chain comprises a constant region of a human immunoglobulin κ light chain.

[0044]

(28) The antibody according to (27), wherein the heavy chain comprises a constant region of a human immunoglobulin G1 heavy chain.

[0045]

(29) The antibody according to (28), wherein in the human immunoglobulin G1 heavy chain, leucine (Leu) at position 234 is substituted by alanine (Ala), and leucine (Leu) at position 235 is substituted by alanine (Ala).

[0046]

(30) The antibody according to (27), wherein the heavy chain comprises a constant region of a human immunoglobulin G2 heavy chain.

[0047]

(31) The antibody according to (27), wherein the heavy chain comprises a constant region of a human immunoglobulin G4 heavy chain.

[0048]

(32) The antibody according to (31), wherein in the human immunoglobulin G4 heavy chain, serine (Ser) at position 241 is substituted by proline (Pro).

[0049]

(33) An antibody or an antigen-binding fragment thereof which specifically binds to an extracellular region of ALK2 protein and inhibits ALK2-mediated BMP signal transduction, wherein the antibody or the antigen-binding fragment comprises

(a) a heavy chain variable region sequence selected from the group consisting of the following amino acid sequences:

a1) an amino acid sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 28;

a2) an amino acid sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 30;

a3) an amino acid sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 105;

a4) an amino acid sequence having at least 95% identity to any of the amino acid sequences a1) to a3);

a5) an amino acid sequence having at least 99% identity to any of the amino acid sequences a1) to a3); and

a6) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in any of the amino acid sequences a1) to a3); and

(b) a light chain variable region sequence selected from the group consisting of the following amino acid sequences:

b1) an amino acid sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 32;

b2) an amino acid sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 34;

b3) an amino acid sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 36;

b4) an amino acid sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 38;

b5) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences b1) to b4);

b6) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences b1) to b4); and

b7) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in any one amino acid sequence selected from the amino acid sequences b1) to b4).

[0050]

(34) The antibody or the antigen-binding fragment thereof according to (33), wherein the antibody is an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 30 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 36, or an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 105

and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 36.

[0051]

(35) The antibody according to (33), wherein the antibody is an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 472 of the amino acid sequence of SEQ ID NO: 30 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 36, or an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 468 of the amino acid sequence of SEQ ID NO: 105 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 36.

[0052]

(36) The antibody according to (33), wherein the antibody is an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 468 of the amino acid sequence of SEQ ID NO: 105 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 36.

[0053]

(37) An antibody or an antigen-binding fragment thereof which specifically binds to an extracellular region of ALK2 protein and inhibits ALK2-mediated BMP signal transduction, wherein the antibody comprises

(a) a heavy chain variable region sequence selected from the group consisting of the following amino acid sequences:

a1) an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 40;

a2) an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 42;

a3) an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 44;

- a4) an amino acid sequence consisting of the amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 46;
- a5) an amino acid sequence consisting of amino acid numbers 20 to 140 of an amino acid sequence of SEQ ID NO: 48;
- a6) an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 107;
- a7) an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 109;
- a8) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences a1) to a7);
- a9) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences a1) to a7); and
- a10) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in any one amino acid sequence selected from the amino acid sequences a1) to a7); and
- (b) a light chain variable region sequence selected from the group consisting of the following amino acid sequences:
 - b1) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 50;
 - b2) an amino acid sequence consisting of amino acid numbers 21 to 129 of an amino acid sequence of SEQ ID NO: 52;
 - b3) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 54;
 - b4) an amino acid sequence consisting of amino acid numbers 21 to 129 of an amino acid sequence of SEQ ID NO: 56;
 - b5) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 58;
 - b6) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences b1) to b5);

b7) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences b1) to b5); and

b8) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in any one amino acid sequence selected from the amino acid sequences b1) to b5).

[0054]

(38) The antibody or the antigen-binding fragment thereof according to (37), wherein the antibody is an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 42 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 52, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 44 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 56, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 107 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 52, or an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 109 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 56.

[0055]

(39) The antibody according to (37), wherein the antibody is an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 42 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 52, an antibody consisting of a heavy chain comprising an amino acid sequence

consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 44 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 56, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 107 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 52, or an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 109 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 56.

[0056]

(40) The antibody according to (37), wherein the antibody is an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 107 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 52, or an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 109 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 56.

[0057]

(41) An antibody or an antigen-binding fragment thereof which specifically binds to an extracellular region of ALK2 protein and inhibits ALK2-mediated BMP signal transduction, wherein the antibody comprises

(a) a heavy chain variable region sequence selected from the group consisting of the following amino acid sequences:

a1) an amino acid sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 111;

a2) an amino acid sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 113;

- a3) an amino acid sequence having at least 95% identity to the amino acid sequence a1) or a2);
- a4) an amino acid sequence having at least 99% identity to the amino acid sequence a1) or a2);
- and
- a5) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in the amino acid sequence a1) or a2); and
- (b) a light chain variable region sequence selected from the group consisting of the following amino acid sequences:
 - b1) an amino acid sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 115;
 - b2) an amino acid sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 117;
 - b3) an amino acid sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 119;
 - b4) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences b1) to b3);
 - b5) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences b1) to b3); and
 - b6) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in any one amino acid sequence selected from the amino acid sequences b1) to b3).

[0058]

(42) An antibody or an antigen-binding fragment thereof which specifically binds to an extracellular region of ALK2 protein and inhibits ALK2-mediated BMP signal transduction, wherein the antibody comprises

- (a) a heavy chain variable region sequence selected from the group consisting of the following amino acid sequences:
 - a1) an amino acid sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 121;

a2) an amino acid sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 123;

a3) an amino acid sequence having at least 95% identity to the amino acid sequence a1) or a2);

a4) an amino acid sequence having at least 99% identity to the amino acid sequence a1) or a2);

and

a5) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in the amino acid sequence a1) or a2); and

(b) a light chain variable region sequence selected from the group consisting of the following amino acid sequences:

b1) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 125;

b2) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 127;

b3) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 129;

b4) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences b1) to b3);

b5) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences b1) to b3); and

b6) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in any one amino acid sequence selected from the amino acid sequences b1) to b3).

[0059]

(43) The antibody according to any one of (1) to (42), wherein the antibody is an antibody comprising a heavy chain in which one to several carboxyl-terminal amino acids are deleted.

[0060]

(44) The antibody or the antigen-binding fragment thereof according to any one of (1) to (43), wherein the antibody is an antibody having a pyroglutamylated amino-terminal amino acid residue in a heavy or light chain thereof.

[0061]

(45) A pharmaceutical composition comprising at least one antibody or antigen-binding fragment according to any one of (1) to (44).

[0062]

(46) The pharmaceutical composition according to (45), wherein the pharmaceutical composition is a therapeutic and/or prophylactic drug for ectopic ossification.

[0063]

(47) A pharmaceutical composition for the treatment and/or prevention of ectopic ossification, comprising at least any one antibody or antigen-binding fragment thereof according to any one of (1) to (44) and at least any one drug selected from the group consisting of anti-inflammatory drugs, steroids, bisphosphonates, muscle relaxants, and retinoic acid receptor (RAR) γ agonists.

[0064]

(48) The pharmaceutical composition according to (46) or (47), wherein the ectopic ossification is fibrodysplasia ossificans progressiva (FOP), progressive osseous heteroplasia (POH), traumatic ectopic ossification, or ectopic ossification after implant arthroplasty.

[0065]

(49) The pharmaceutical composition according to (46) or (47), wherein the ectopic ossification is fibrodysplasia ossificans progressiva (FOP).

[0066]

(50) The pharmaceutical composition according to (45), wherein the pharmaceutical composition is a therapeutic and/or prophylactic drug for anemia.

[0067]

(51) The pharmaceutical composition according to (45), wherein the pharmaceutical composition is a therapeutic and/or prophylactic drug for diffuse intrinsic pontine glioma (DIPG).

[0068]

(52) A method for treating and/or preventing ectopic ossification, comprising administering at least one antibody or antigen-binding fragment according to any one of (1) to (44), or at least one pharmaceutical composition according to any one of (45) to (49).

[0069]

(53) A method for treating and/or preventing ectopic ossification, comprising administering at least one antibody or antigen-binding fragment thereof according to any one of (1) to (44), or at least one pharmaceutical composition according to any one of (45) to (49), and at least any one drug selected from the group consisting of anti-inflammatory drugs, steroids, bisphosphonates, muscle relaxants, and retinoic acid receptor (RAR) γ agonists, concurrently (or, at the same time) or separately (or, one after another).

[0070]

(54) The method according to (52) or (53), wherein the ectopic ossification is fibrodysplasia ossificans progressiva (FOP), progressive osseous heteroplasia (POH), traumatic ectopic ossification, or ectopic ossification after implant arthroplasty.

[0071]

(55) The method according to (52) or (53), wherein the ectopic ossification is fibrodysplasia ossificans progressiva (FOP).

[0072]

(56) A method for treating and/or preventing anemia, comprising administering at least one antibody or antigen-binding fragment according to any one of (1) to (44), or at least one pharmaceutical composition according to (45) or (50).

[0073]

(57) A method for treating and/or preventing diffuse intrinsic pontine glioma (DIPG), comprising administering at least one antibody or antigen-binding fragment according to any one of (1) to (44), or at least one pharmaceutical composition according to (45) or (51).

[0074]

(58) A polynucleotide encoding an antibody according to any one of (1) to (44).

[0075]

(59) A polynucleotide comprising

(a) a polynucleotide selected from the group consisting of the following nucleotide sequences:

a1) a nucleotide sequence consisting of nucleotide numbers 58 to 426 of the nucleotide sequence of SEQ ID NO: 27;

a2) a nucleotide sequence consisting of nucleotide numbers 58 to 426 of the nucleotide sequence of SEQ ID NO: 29;

a3) a nucleotide sequence consisting of nucleotide numbers 58 to 426 of the nucleotide sequence of SEQ ID NO: 104;

a4) a nucleotide sequence having at least 95% identity to any of the nucleotide sequences a1) to a3);

a5) a nucleotide sequence having at least 99% identity to any of the nucleotide sequences a1) to a3);

a6) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any of the nucleotide sequences a1) to a3); and

a7) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in the nucleotide sequence a1) or a2); and/or

(b) a polynucleotide selected from the group consisting of the following nucleotide sequences:

b1) a nucleotide sequence consisting of nucleotide numbers 86 to 424 of the nucleotide sequence of SEQ ID NO: 31;

b2) a nucleotide sequence consisting of nucleotide numbers 86 to 424 of the nucleotide sequence of SEQ ID NO: 33;

b3) a nucleotide sequence consisting of nucleotide numbers 86 to 424 of the nucleotide sequence of SEQ ID NO: 35;

b4) a nucleotide sequence consisting of nucleotide numbers 86 to 424 of the nucleotide sequence of SEQ ID NO: 37;

b5) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b4);

b6) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b4);

b7) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences b1) to b4); and

b8) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences b1) to b4).

[0076]

(60) A polynucleotide comprising

(a) a polynucleotide selected from the group consisting of the following nucleotide sequences:

a1) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 39;

a2) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 41;

a3) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 43;

a4) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 45;

a5) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 47;

a6) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 106;

a7) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 108;

a8) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences a1) to a7);

a9) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences a1) to a7);

a10) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences a1) to a7); and

a11) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences a1) to a7); and/or

(b) a polynucleotide selected from the group consisting of the following nucleotide sequences:

b1) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 49;

b2) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 51;

b3) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 53;

b4) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 55;

b5) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 57;

b6) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b5);

b7) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b5);

b8) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences b1) to b5); and

b9) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences b1) to b5).

[0077]

(61) A polynucleotide comprising

- (a) a polynucleotide selected from the group consisting of the following nucleotide sequences:
- a1) a nucleotide sequence consisting of nucleotide numbers 58 to 411 of the nucleotide sequence of SEQ ID NO: 110;
 - a2) a nucleotide sequence consisting of nucleotide numbers 58 to 411 of the nucleotide sequence of SEQ ID NO: 112;
 - a3) a nucleotide sequence having at least 95% identity to the nucleotide sequence a1) or a2);
 - a4) a nucleotide sequence having at least 99% identity to the nucleotide sequence a1) or a2);
 - a5) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to the nucleotide sequence a1) or a2); and
 - a6) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in the nucleotide sequence a1) or a2); and/or
- (b) a polynucleotide selected from the group consisting of the following nucleotide sequences:
- b1) a nucleotide sequence consisting of nucleotide numbers 61 to 384 of the nucleotide sequence of SEQ ID NO: 114;
 - b2) a nucleotide sequence consisting of nucleotide numbers 61 to 384 of the nucleotide sequence of SEQ ID NO: 116;
 - b3) a nucleotide sequence consisting of nucleotide numbers 61 to 384 of the nucleotide sequence of SEQ ID NO: 118;
 - b4) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b3);
 - b5) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b3);
 - b6) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences b1) to b3); and
 - b7) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences b1) to b3).

[0078]

(62) A polynucleotide comprising

(a) a polynucleotide selected from the group consisting of the following nucleotide sequences:

a1) a nucleotide sequence consisting of nucleotide numbers 58 to 411 of the nucleotide sequence of SEQ ID NO: 120;

a2) a nucleotide sequence consisting of nucleotide numbers 58 to 411 of the nucleotide sequence of SEQ ID NO: 122;

a3) a nucleotide sequence having at least 95% identity to the nucleotide sequence a1) or a2);

a4) a nucleotide sequence having at least 99% identity to the nucleotide sequence a1) or a2);

a5) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to the nucleotide sequence a1) or a2); and

a6) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in the nucleotide sequence a1) or a2); and/or

(b) a polynucleotide selected from the group consisting of the following nucleotide sequences:

b1) a nucleotide sequence consisting of nucleotide numbers 61 to 387 of the nucleotide sequence of SEQ ID NO: 124;

b2) a nucleotide sequence consisting of nucleotide numbers 61 to 387 of the nucleotide sequence of SEQ ID NO: 126;

b3) a nucleotide sequence consisting of nucleotide numbers 61 to 387 of the nucleotide sequence of SEQ ID NO: 128;

b4) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b3);

b5) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b3);

b6) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences b1) to b3); and

b7) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences b1) to b3).

[0079]

(63) A vector comprising any one polynucleotide according to any one of (58) to (62).

[0080]

(64) A transformed host cell comprising any one polynucleotide according to any one of (58) to (62).

[0081]

(65) A transformed host cell comprising a vector according to (63).

[0082]

(66) A method for producing an antibody according to any one of (1) to (44), comprising the step of culturing a host cell according to (64) or (65) and purifying the antibody from the culture product.

[0083]

According to the present invention, a therapeutic and/or prophylactic drug for ectopic ossification and/or bone dysplasia can be obtained.

[0084]

The present specification encompasses the contents disclosed in Japanese Patent Application No. 2015-017882 from which the present application claims the priority.

BRIEF DESCRIPTION OF DRAWINGS

[0085]

[Figure 1] This figure is a graph showing the recognition of mouse ALK2-His&Fc ("mALK2-Fc") by monoclonal antibodies produced by hybridomas A2-11E, A2-15A, A2-25C, and A2-27D, and the binding of these monoclonal antibodies to human Fc. A medium was used as a control ("Control medium").

[Figure 2A] This figure is a graph showing that the monoclonal antibodies produced by the hybridomas A2-11E, A2-15A, A2-25C, and A2-27D do not recognize cells expressing a fluorescent protein EGFP.

[Figure 2B] This figure is a graph showing that the monoclonal antibodies produced by the hybridomas A2-11E, A2-15A, A2-25C, and A2-27D recognize cells expressing wild-type mouse ALK2 (mALK2(WT)-EGFP).

[Figure 2C] This figure is a graph showing that the monoclonal antibodies produced by the hybridomas A2-11E, A2-15A, A2-25C, and A2-27D recognize cells expressing wild-type human ALK2 (hALK2(WT)-EGFP).

[Figure 2D] This figure is a graph showing that the monoclonal antibodies produced by the hybridomas A2-11E, A2-15A, A2-25C, and A2-27D recognize cells expressing human ALK2 having a FOP mutation (R206H) (hALK2(R206H)-EGFP).

[Figure 3] This figure is a graph showing that the monoclonal antibodies produced by the hybridomas A2-11E, A2-15A, A2-25C, and A2-27D specifically recognize only ALK2-expressing cells and do not recognize cells expressing ALK1, ALK3, or ALK6.

[Figure 4] This figure is a graph showing that the monoclonal antibodies produced by the hybridomas A2-11E, A2-15A, A2-25C, and A2-27D recognize cells expressing wild-type ALK2 and cells expressing the shown 12 types of ALK2 mutants identified in FOP.

[Figure 5] This figure is a graph showing that the monoclonal antibodies produced by the hybridomas A2-11E, A2-15A, A2-25C, and A2-27D inhibit, in a dose-dependent manner, BMP-specific luciferase (luc) activity induced by BMP7 in HEK293A cells in which wild-type ALK2 or a R206H mutant has been overexpressed.

[Figure 6] This figure is a graph showing that the monoclonal antibodies produced by the hybridomas A2-11E, A2-15A, A2-25C, and A2-27D cannot completely inhibit the BMP2-induced differentiation of C2C12 cells into osteoblast-like cells.

[Figure 7] This figure is a graph showing that the monoclonal antibodies produced by the hybridomas A2-11E, A2-15A, A2-25C, and A2-27D inhibit, in a dose-dependent manner, the BMP7- or GDF2/BMP9-induced differentiation of C2C12 cells into osteoblast-like cells.

[Figure 8] This figure is a graph showing that the monoclonal antibodies produced by the hybridomas A2-15A and A2-27D inhibit BMP7- or GDF2/BMP9-induced ectopic osteoinduction in mouse skeletal muscle tissues.

[Figure 9] This figure is a graph showing that chimeric antibodies cA2-15A and cA2-27D exhibit inhibitory activity equivalent to the rat monoclonal antibodies A2-15A and A2-27D, respectively, against BMP-specific luciferase (luc) activity induced by BMP7.

[Figure 10] This figure is a graph showing that the chimeric antibodies cA2-15A and cA2-27D inhibit, in a dose-dependent manner, the BMP-induced differentiation of C2C12 cells into osteoblast-like cells.

[Figure 11] This figure shows the amino acid sequence of each CDR sequence of the A2-15A antibody.

[Figure 12] This figure shows the amino acid sequence of each CDR sequence of the A2-27D antibody.

[Figure 13] This figure shows the amino acid sequence of each CDR sequence of the A2-11E antibody.

[Figure 14] This figure shows the amino acid sequence of each CDR sequence of the A2-25C antibody.

[Figure 15] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-15A-H1.

[Figure 16] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-15A-H4.

[Figure 17] This figure shows the nucleotide sequence of a DNA fragment comprising sequences encoding humanized hA2-15A-L1, and the amino acid sequence thereof.

[Figure 18] This figure shows the nucleotide sequence of a DNA fragment comprising sequences encoding humanized hA2-15A-L4, and the amino acid sequence thereof.

[Figure 19] This figure shows the nucleotide sequence of a DNA fragment comprising sequences encoding humanized hA2-15A-L6, and the amino acid sequence thereof.

[Figure 20] This figure shows the nucleotide sequence of a DNA fragment comprising sequences encoding humanized hA2-15A-L7, and the amino acid sequence thereof.

[Figure 21] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-27D-H1.

[Figure 22] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-27D-H2.

[Figure 23] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-27D-H3.

[Figure 24] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-27D-H4.

[Figure 25] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-27D-H5.

[Figure 26] This figure shows the nucleotide sequence of a DNA fragment comprising sequences encoding humanized hA2-27D-L1, and the amino acid sequence thereof.

[Figure 27] This figure shows the nucleotide sequence of a DNA fragment comprising sequences encoding humanized hA2-27D-L2, and the amino acid sequence thereof.

[Figure 28] This figure shows the nucleotide sequence of a DNA fragment comprising sequences encoding humanized hA2-27D-L3, and the amino acid sequence thereof.

[Figure 29] This figure shows the nucleotide sequence of a DNA fragment comprising sequences encoding humanized hA2-27D-L4, and the amino acid sequence thereof.

[Figure 30] This figure shows the nucleotide sequence of a DNA fragment comprising sequences encoding humanized hA2-27D-L5, and the amino acid sequence thereof.

[Figure 31] This figure is a graph showing that a humanized A2-15A antibody (IgG1) and humanized A2-27D antibodies (IgG1) inhibit, in a dose-dependent manner, BMP-specific luciferase (luc) activity induced by BMP7.

[Figure 32] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-15A-H4 IgG2 type.

[Figure 33] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-27D-H2-LALA.

[Figure 34] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-27D-H3-LALA.

[Figure 35] This figure is a graph showing that a humanized A2-15A antibody (IgG2) and a humanized A2-27D antibody (LALA) inhibit, in a dose-dependent manner, BMP-specific luciferase (luc) activity induced by BMP7.

[Figure 36] This figure is a graph showing that the humanized A2-15A antibody (IgG2) and the humanized A2-27D antibody (LALA) inhibit BMP7-induced ectopic osteoinduction in mouse skeletal muscle tissues.

[Figure 37] This figure shows the X-ray crystal structure of a complex of human ALK2-ECD and human chimeric cA2-27D-Fab.

[Figure 38] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-11E-H3.

[Figure 39] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-11E-H4.

[Figure 40] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-11E-L2.

[Figure 41] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-11E-L3.

[Figure 42] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-11E-L4.

[Figure 43] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-25C-H3.

[Figure 44] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-25C-H4.

[Figure 45] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-25C-L1.

[Figure 46] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-25C-L2.

[Figure 47] This figure shows the nucleotide sequence and amino acid sequence of humanized hA2-25C-L3.

[Figure 48] This figure is a graph showing that humanized A2-11E antibodies (IgG1) and humanized A2-25C antibodies (IgG1) inhibit, in a dose-dependent manner, the BMP-induced differentiation of C2C12 cells into osteoblast-like cells.

[Figure 49] This figure shows the X-ray crystal structure of a complex of human ALK2-ECD and human chimeric cA2-25C-Fab.

[Figure 50] This figure is a graph showing that the monoclonal antibody produced by the hybridoma A2-27D inhibits BMP7-induced luciferase (Luc) activity in wild-type ALK2 (WT) and all of the shown 13 types of mutants.

MODES FOR CARRYING OUT THE INVENTION

[0086]

1. Definition

As used herein, the term "gene" includes not only DNA but mRNA, cDNA, and cRNA.

[0087]

As used herein, the term "polynucleotide" is used with the same meaning as a nucleic acid and also includes DNA, RNA, probes, oligonucleotides, and primers.

[0088]

As used herein, the "polypeptide" and the "protein" are used interchangeably with each other.

[0089]

As used herein, the "RNA fraction" refers to a fraction containing RNA.

[0090]

As used herein, the "cell" also includes cells within animal individuals and cultured cells.

[0091]

As used herein, "ALK2" is used with the same meaning as ALK2 protein and includes wild-type ALK2 and mutants (also referred to as mutant ALK2).

[0092]

As used herein, the "antigen-binding fragment of an (the) antibody", also called "functional fragment of an (the) antibody", means a partial fragment of the antibody having an activity binding to the antigen and includes, for example, Fab, F(ab')₂, Fv, scFv, diabodies, linear antibodies, and multispecific antibodies formed from antibody fragments. The antigen-binding fragment of an antibody also includes Fab', which is a monovalent fragment of antibody variable regions obtained by treatment of F(ab')₂ under reducing conditions. However, the antigen-binding fragment of an antibody is not limited to these molecules as long as the antigen-binding fragment has the ability to bind to the antigen. Such an antigen-binding fragment includes not only a fragment obtained by treating a full-length molecule of the antibody protein with an appropriate enzyme but a protein produced in appropriate host cells using a genetically engineered antibody gene.

[0093]

As used herein, the "epitope", also called "antigenic determinant", generally refers to an antibody-binding antigenic site consisting of at least 7 amino acids, at least 8 amino acids, at least 9 amino acids, or at least 10 amino acids, of an antigen. As used herein, the epitope means a partial peptide or a partial conformation of ALK2 to which a particular anti-ALK2 antibody binds. The epitope as a partial peptide of ALK2 can be determined by a method well known to those skilled in the art such as immunoassay and can be determined, for example, by the following method: various partial structures of ALK2 are prepared. For the preparation of the partial structures, an oligopeptide synthesis technique known in the art can be used. For example, a series of polypeptide fragments having an appropriate length are prepared in order from the C or N terminus of ALK2 by use of a gene recombination technique well known to those skilled in the art. Then, the reactivity of the antibody therewith is studied to roughly determine a recognition site. Then, shorter peptides are synthesized, and the reactivity of the antibody with these peptides can be studied to determine the epitope. Alternatively, the epitope as a partial conformation of ALK2 to which a particular ALK2 antibody binds can be determined by identifying amino acid residues of ALK2 adjacent to the antibody by X-ray crystal structure analysis. Provided that a second anti-ALK2 antibody binds to a partial peptide or a partial conformation that is bound by a first

anti-ALK2 antibody, the first antibody and the second antibody can be determined to share an epitope. If the specific sequence or structure of an epitope is not determined, the first antibody and the second antibody can be determined to share the epitope by confirming that the second anti-ALK2 antibody (cross-)competes with the first anti-ALK2 antibody for binding to ALK2 (i.e., that the second antibody interferes with the binding of the first antibody to ALK2). When the first antibody and the second antibody bind to a common epitope and the first antibody has an activity such as inhibitory activity against ALK2-mediated BMP signals, the second antibody can also be expected to have similar activity.

[0094]

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

[0095]

In the present invention, the wording "hybridizing under stringent conditions" means hybridization under conditions involving hybridization at approximately 50 to 70°C (e.g., 68°C) in a commercially available hybridization solution ExpressHyb Hybridization Solution (manufactured by Clontech Laboratories, Inc.), or hybridization at approximately 50 to 70°C (e.g., 68°C) in the presence of approximately 0.7 to 1.0 M NaCl using a DNA-immobilized filter, followed by washing at approximately 50 to 70°C (e.g., 68°C) using an SSC solution having an approximately 0.1 to 2 × concentration (SSC having a 1 × concentration consists of 150 mM NaCl and 15 mM sodium citrate; if necessary, the solution may contain

approximately 0.1 to 0.5% SDS) which permits identification, or hybridization under conditions equivalent thereto.

[0096]

As used herein, the term "several" in the phrase "one to several" and "one or several" refers to 2 to 10. The term "several" is preferably 10 or less, more preferably 5 or 6 or less, further preferably 2 or 3.

[0097]

2. ALK2

The ALK2 gene is a responsible gene for FOP encoding a receptor of BMP that induces ectopic bone formation in soft tissues including skeletal muscle tissues. Mutant ALK2 having amino acid substitutions has been found from familial and sporadic FOP cases. For example, L196P (mutation that substitutes leucine at position 196 by proline), delP197_F198insL (mutation that deletes proline at position 197 and phenylalanine at position 198 and inserts leucine), R202I (mutation that substitutes arginine at position 202 by isoleucine), R206H (mutation that substitutes arginine at position 206 by histidine), Q207E (mutation that substitutes glutamine at position 207 by glutamic acid), R258S (mutation that substitutes arginine at position 258 by serine), R258G (mutation that substitutes arginine at position 258 by glycine), G325A (mutation that substitutes glycine at position 325 by alanine), G328E (mutation that substitutes glycine at position 328 by glutamic acid), G328R (mutation that substitutes glycine at position 328 by arginine), G328W (mutation that substitutes glycine at position 328 by tryptophan), G356D (mutation that substitutes glycine at position 356 by aspartic acid), and R375P (mutation that substitutes arginine at position 375 by proline) mutants are known as mutants of human ALK2.

[0098]

ALK2 used in the present invention can be obtained by *in vitro* synthesis or by production from host cells through gene manipulation. Specifically, ALK2 cDNA is inserted into a vector that permits expression. Then, the ALK2 protein can be obtained by synthesis in a solution containing enzymes, substrates, and energy substances necessary for transcription

and translation, or by expression from other prokaryotic or eukaryotic host cells transformed with the vector.

[0099]

ALK2 used in the present invention is ALK2 derived from a mammal including a human or a mouse. For example, the amino acid sequence and nucleotide sequence of human ALK2 are available with reference to GenBank Accession No. NM-001105. Herein, the amino acid sequence is also disclosed as SEQ ID NO: 84, and the nucleotide sequence is disclosed as SEQ ID NO: 85. The amino acid sequence and nucleotide sequence of mouse ALK2 are available with reference to GenBank Accession No. NP-001103674. Herein the amino acid sequence is also disclosed as SEQ ID NO: 86, and the nucleotide sequence is disclosed as SEQ ID NO: 87. ALK2 is also called ACVR1 (activin A type I receptor 1) or ACTR1 (activin receptor type 1), and all of these terms represent the same molecules.

[0100]

The ALK2 cDNA can be obtained by a so-called PCR method which involves carrying out polymerase chain reaction (hereinafter, referred to as "PCR") (Saiki, R.K., et al., Science, (1988) 239, 487-49), for example, with a cDNA library expressing the ALK2 cDNA as a template using primers specifically amplifying the ALK2 cDNA.

[0101]

The ALK2 cDNA also includes a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to the nucleotide sequence encoding human or mouse ALK2 and encodes a protein having biological activity equivalent to ALK2. The ALK2 cDNA further includes a splicing variant that has been transcribed from the human or mouse ALK2 gene locus or a polynucleotide that hybridizes under stringent conditions thereto, and encodes a protein having biological activity equivalent to ALK2.

[0102]

ALK2 also includes a protein that consists of an amino acid sequence derived from the amino acid sequence of human or mouse ALK2 or an amino acid sequence thereof free from the signal sequence by the substitution, deletion, or addition of one or several amino acids, and

has biological activity equivalent to ALK2. ALK2 further includes a protein that consists of an amino acid sequence encoded by a splicing variant transcribed from the human or mouse ALK2 gene locus, or an amino acid sequence derived from this amino acid sequence by the substitution, deletion, or addition of one or several amino acids, and has biological activity equivalent to ALK2.

[0103]

3. Detection of ectopic ossification and/or bone dysplasia

Ectopic ossification and/or bone dysplasia is induced by ALK2-mediated BMP signal transduction.

[0104]

The "ectopic ossification" means bone formation at a site where the bone is originally absent. The "bone dysplasia" means the abnormal shape or quality of the existing bone. Examples of the "ectopic ossification" can include, but are not limited to, fibrodysplasia ossificans progressiva (FOP), progressive osseous heteroplasia (POH), traumatic ectopic ossification, and ectopic ossification after implant arthroplasty. Examples of the "bone dysplasia" can include, but are not limited to, spondyloarthritis (SpA) and ankylosing spondylitis (AS).

[0105]

ALK2 is a transmembrane serine-threonine kinase receptor binding to BMP. ALK2 binds to BMP at the N-terminal extracellular region and activates a downstream intracellular signal transduction system through intracellular serine-threonine kinase. Bone morphogenetic protein (BMP) is a multifunctional growth factor belonging to the transforming growth factor β (TGF- β) superfamily, and approximately 20 BMP family members have been identified. BMP has been confirmed to induce ectopic bone formation in soft tissues including skeletal muscle tissues and is therefore considered to participate in diseases promoting abnormal bone formation. BMP-2 and BMP-4 are considered to have higher affinity for ALK3 than that for ALK2. Since ALK3 is expressed ubiquitously as compared with ALK2, BMP-2 or BMP-4 seems to be often used in general in experiments of inducing ectopic ossification at various sites. On the other hand, BMP-7 has relatively high affinity

for ALK2. BMP-9 is generally considered to have high affinity for ALK1 and has also been found to have relatively high affinity for ALK2. In FOP, ectopic ossification occurs via ALK2. Therefore, the presence or absence of therapeutic and/or prophylactic effects on FOP can probably be confirmed by testing efficacy for ectopic osteoinduction caused by the activation of ALK2-mediated signals by BMP-7 and BMP-9.

[0106]

The culture of myoblasts (C2C12 cells) in the presence of BMP suppresses their differentiation into mature muscle cells through an intracellular signal transduction mechanism specific for BMP and instead induces the differentiation into osteoblasts. Thus, ALK2-mediated BMP signal transduction can be analyzed with models of induction of differentiation of C2C12 cells into osteoblasts by BMP.

[0107]

4. Production of anti-ALK2 antibody

The antibody of the present invention against ALK2 can be obtained by a method described in, for example, WO2009/091048, WO2011/027808, or WO2012/133572. Specifically, nonhuman animals are immunized with the antigen of interest. Lymphs, lymph tissues, blood samples, or bone marrow-derived cells are collected from the animals after establishment of immunity. Plasma cells and/or plasmablasts of the nonhuman animals specifically binding to the antigen of interest are selected. A gene of an antibody to the antigen of interest is collected from the obtained plasma cells and/or plasmablasts, and its nucleotide sequence is identified. The antibody or a fragment thereof can be obtained on the basis of the identified nucleotide sequence of the gene. Alternatively, the antibody or the antibody fragment can be obtained by obtaining plasma cells and/or plasmablasts in the same way as above from the blood of a human infected patient. The obtained antibody can be tested for its binding activity to ALK2 to select an antibody applicable to human diseases. Examples of the monoclonal antibody thus obtained can include A2-11E, A2-15A, A2-25C, and A2-27D.

[0108]

Herein, amino acid numbers assigned to CDR/FR characteristic of an antibody are laid out according to the KABAT numbering (KABAT et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service National Institutes of Health, Bethesda, MD. (1991)).

[0109]

The monoclonal antibody can also be obtained 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)) by fusing antibody-producing cells that produce the antibody against ALK2 with myeloma cells to establish hybridomas. Specific examples of such a method are described in WO2009/48072 (published on April 16, 2009) and WO2010/117011 (published on October 14, 2010). However, the method for obtaining monoclonal antibodies corresponds to an already established field and is not limited to the specific examples described above.

[0110]

The antibody of the present invention includes monoclonal antibodies against ALK2 described above as well as, for example, polyclonal antibodies similarly having therapeutic and/or prophylactic effects, recombinant antibodies artificially engineered for the purpose of, for example, reducing heterogeneous antigenicity against humans, for example, chimeric antibodies, humanized antibodies, human antibodies, and the like. These antibodies can be produced by use of known methods.

[0111]

Examples of the chimeric antibody can include chimeric antibodies comprising variable regions and constant regions (Fc) of antibodies derived from different species, for example, the variable regions of a mouse- or rat-derived antibody joined to human-derived constant regions (see Proc. Natl. Acad. Sci. U.S.A., 81, 6851-6855, (1984)). One example of the chimeric antibody derived from A2-15A can include an antibody consisting of a heavy chain having an amino acid sequence consisting of amino acid numbers 20 to 472 of SEQ ID NO: 20 of the Sequence Listing and a light chain having an amino acid sequence consisting of amino acids 21 to 237 of SEQ ID NO: 22. One example of the chimeric antibody derived from A2-

27D can include an antibody consisting of a heavy chain having an amino acid sequence consisting of amino acids 20 to 470 of SEQ ID NO: 24 of the Sequence Listing and a light chain having an amino acid sequence consisting of amino acids 21 to 234 of SEQ ID NO: 26.

[0112]

Examples of the humanized antibody can include an antibody comprising CDRs alone integrated into a human-derived antibody (see Nature (1986) 321, p. 522-525), and an antibody comprising the CDR sequences as well as amino acid residues of a portion of frameworks grafted into a human antibody by a CDR grafting method (International Publication No. WO90/07861).

[0113]

The humanized antibody derived from the A2-15A antibody is included in the antibody of the present invention as long as the humanized antibody contains all of the 6 CDR sequences of A2-15A and has binding activity against ALK2. The heavy chain variable region of the A2-15A antibody comprises CDRH1 consisting of the amino acid sequence of SEQ ID NO: 59 (GFTFSHYMA), CDRH2 consisting of the amino acid sequence of SEQ ID NO: 60 (SITNSGGSINYRDSVKG), and CDRH3 consisting of the amino acid sequence of SEQ ID NO: 61 (EGGENYGGYPPFAY). The light chain variable region of the A2-15A antibody comprises CDRL1 consisting of the amino acid sequence of SEQ ID NO: 62 (RANQGVSLSRYNLMH), CDRL2 consisting of the amino acid sequence of SEQ ID NO: 63 (RSSNLAS), and CDRL3 consisting of the amino acid sequence of SEQ ID NO: 64 (QQSRESPFT). The amino acid sequences of these CDRs are also described in Figure 11.

[0114]

The humanized antibody derived from the A2-27D antibody is included in the antibody of the present invention as long as the humanized antibody contains all of the 6 CDR sequences of A2-27D and has binding activity against ALK2. The heavy chain variable region of the A2-27D antibody comprises CDRH1 consisting of the amino acid sequence of SEQ ID NO: 65 (GSTFSNYGMK), CDRH2 consisting of the amino acid sequence of SEQ ID NO: 66 (SIRSSTYIYYADTVKG), and CDRH3 consisting of the amino acid sequence of SEQ ID NO: 67 (AISTPFYWYFDF). The light chain variable region of the A2-27D

antibody comprises CDRL1 consisting of the amino acid sequence of SEQ ID NO: 68 (LASSSVSYMT), CDRL2 consisting of the amino acid sequence of SEQ ID NO: 69 (GTSNLAS), and CDRL3 consisting of the amino acid sequence of SEQ ID NO: 70 (LHLTSYPPYT). The amino acid sequences of these CDRs are also described in Figure 12.

[0115]

The humanized antibody derived from the A2-11E antibody is included in the antibody of the present invention as long as the humanized antibody contains all of the 6 CDR sequences of A2-11E and has binding activity against ALK2. The heavy chain variable region of the A2-11E antibody comprises CDRH1 consisting of the amino acid sequence of SEQ ID NO: 72 (GFTFSNYMY), CDRH2 consisting of the amino acid sequence of SEQ ID NO: 73 (SINTDGGSTYYPDSVKG), and CDRH3 consisting of the amino acid sequence of SEQ ID NO: 74 (STPNIPLAY). The light chain variable region of the A2-11E antibody comprises CDRL1 consisting of the amino acid sequence of SEQ ID NO: 75 (KASQNIYKYLN), CDRL2 consisting of the amino acid sequence of SEQ ID NO: 76 (YSNSLQT), and CDRL3 consisting of the amino acid sequence of SEQ ID NO: 77 (FQYSSGPT). The amino acid sequences of these CDRs are also described in Figure 13.

[0116]

The humanized antibody derived from the A2-25C antibody is included in the antibody of the present invention as long as the humanized antibody contains all of the 6 CDR sequences of A2-25C and has binding activity against ALK2. The heavy chain variable region of the A2-25C antibody comprises CDRH1 consisting of the amino acid sequence of SEQ ID NO: 78 (GFTFSYYAMS), CDRH2 consisting of the amino acid sequence of SEQ ID NO: 79 (SISRGGDNTYYRDTVKG), and CDRH3 consisting of the amino acid sequence of SEQ ID NO: 80 (LNYNNYFDY). The light chain variable region of the A2-25C antibody comprises CDRL1 consisting of the amino acid sequence of SEQ ID NO: 81 (QASQDIGNWLS), CDRL2 consisting of the amino acid sequence of SEQ ID NO: 82 (GATSLAD), and CDRL3 consisting of the amino acid sequence of SEQ ID NO: 83 (LQAYSAPFT). The amino acid sequences of these CDRs are also described in Figure 14.

[0117]

A CDR-engineered humanized antibody prepared by substitution of 1 to 3 amino acid residues in each CDR by other amino acid residues is also included in the antibody of the present invention as long as the humanized antibody has binding activity against ALK2. Examples of the amino acid substitution in CDRL2 can include the substitution of one amino acid of CDRL2 in the amino acid sequence of SEQ ID NO: 34. CDRL2 consisting of the amino acid sequence of SEQ ID NO: 71 (RSSNLAQ) is preferred. The amino acid sequence of this CDR is also described in Figure 11.

[0118]

Actual examples of the humanized antibody derived from the A2-15A antibody can include an arbitrary combination of a heavy chain comprising a heavy chain variable region consisting of an amino acid sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 28 of the Sequence Listing, an amino acid sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 30, or an amino acid sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 105 of the Sequence Listing, and a light chain comprising a light chain variable region consisting of an amino acid sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 32, an amino acid sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 34, an amino acid sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 36, or an amino acid sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 38.

[0119]

Preferred examples of the combination can include an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 28 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 32, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 28 and a light chain comprising a light chain variable region

sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 34, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 142 of an amino acid sequence of SEQ ID NO: 30 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 32, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 30 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 34, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 30 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 36, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 30 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 38, and an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 105 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 36.

[0120]

More preferred examples of the combination can include an antibody consisting of a heavy chain having an amino acid sequence consisting of amino acid numbers 20 to 472 of the amino acid sequence of SEQ ID NO: 28 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 32, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 472 of the amino acid sequence of SEQ ID NO: 28 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 34, an antibody consisting of a heavy chain comprising an

amino acid sequence consisting of amino acid numbers 20 to 472 of the amino acid sequence of SEQ ID NO: 30 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 32, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 472 of the amino acid sequence of SEQ ID NO: 30 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 34, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 472 of the amino acid sequence of SEQ ID NO: 30 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 36, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 472 of the amino acid sequence of SEQ ID NO: 30 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 38, and an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 468 of the amino acid sequence of SEQ ID NO: 105 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 36.

[0121]

Further preferred examples of the combination can include an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 30 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 36, and an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 105 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 36.

[0122]

Still further preferred examples of the combination can include an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 472 of the amino acid sequence of SEQ ID NO: 30 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 36, and an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 468 of the amino acid sequence of SEQ ID NO: 105 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 36.

[0123]

Most preferred examples of the combination can include an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 468 of the amino acid sequence of SEQ ID NO: 105 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 36.

[0124]

Actual examples of the humanized antibody derived from the A2-27D antibody can include an arbitrary combination of a heavy chain comprising a heavy chain variable region consisting of an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 40 of the Sequence Listing, an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 42 of the Sequence Listing, an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 44 of the Sequence Listing, an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 46 of the Sequence Listing, an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 48, an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 107, or an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 109, and a light chain comprising a light chain variable region consisting of an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 50, an

amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 52, an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 54, an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 56, or an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 58.

[0125]

Preferred examples of the combination can include an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 40 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 50, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 40 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 52, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of an amino acid sequence of SEQ ID NO: 40 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 54, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 42 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 50, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of an amino acid sequence of SEQ ID NO: 42 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 52, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of an amino acid sequence of SEQ ID NO: 42 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID

NO: 54, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 44 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 50, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 44 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 52, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of an amino acid sequence of SEQ ID NO: 44 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 54, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 44 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 56, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 46 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 54, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 46 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 56, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 46 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 58, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 48 and a light chain comprising a light chain variable region

sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 56, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 107 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 52, and an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 109 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 56.

[0126]

More preferred examples of the combination can include an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 40 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 50, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 40 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 52, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 40 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 54, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 42 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 50, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 42 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 52, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to

470 of the amino acid sequence of SEQ ID NO: 42 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 54, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 44 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 50, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of an amino acid sequence of SEQ ID NO: 44 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 52, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 44 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 54, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 44 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 56, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 46 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 54, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 46 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 56, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 46 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 58, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 48 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino

acid sequence of SEQ ID NO: 56, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 107 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 52, and an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 109 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 56.

[0127]

Further preferred examples of the combination can include an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 42 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 52, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 44 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 56, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 107 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 52, and an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 109 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 56.

[0128]

Still further preferred examples of the combination can include an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 42 and a light chain comprising an amino acid

sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 52, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 44 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 56, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 107 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 52, and an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 109 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 56.

[0129]

Most preferred examples of the combination can include an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 107 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 52, and an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 109 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 56.

[0130]

Actual examples of the humanized antibody derived from the A2-11E antibody can include an arbitrary combination of a heavy chain comprising a heavy chain variable region consisting of an amino acid sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 111 of the Sequence Listing or an amino acid sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 113, and a light chain comprising a light chain variable region consisting of an amino acid sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 115,

an amino acid sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 117, or an amino acid sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 119.

[0131]

Preferred examples of the combination can include an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 111 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 115, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 111 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 117, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 111 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 128 of an amino acid sequence of SEQ ID NO: 119, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 113 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 115, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 113 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 117, and an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 113 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 119.

[0132]

More preferred examples of the combination can include an antibody consisting of a heavy chain having an amino acid sequence consisting of amino acid numbers 20 to 467 of the amino acid sequence of SEQ ID NO: 111 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 233 of the amino acid sequence of SEQ ID NO: 115, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 467 of the amino acid sequence of SEQ ID NO: 111 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 233 of the amino acid sequence of SEQ ID NO: 117, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 467 of the amino acid sequence of SEQ ID NO: 111 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 233 of the amino acid sequence of SEQ ID NO: 119, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 467 of an amino acid sequence of SEQ ID NO: 113 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 233 of the amino acid sequence of SEQ ID NO: 115, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 467 of the amino acid sequence of SEQ ID NO: 113 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 233 of the amino acid sequence of SEQ ID NO: 117, and an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 467 of the amino acid sequence of SEQ ID NO: 113 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 233 of the amino acid sequence of SEQ ID NO: 119.

[0133]

Actual examples of the humanized antibody derived from the A2-25C antibody can include an arbitrary combination of a heavy chain comprising a heavy chain variable region consisting of an amino acid sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 121 of the Sequence Listing or an amino acid sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 123, and a light chain comprising a light chain variable region consisting of an amino acid sequence

consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 125, an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 127, or an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 129.

[0134]

Preferred examples of the combination can include an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 121 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 125, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 121 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 127, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 121 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 129, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 123 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 125, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 123 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 127, and an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 123 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 129.

[0135]

More preferred examples of the combination can include an antibody consisting of a heavy chain having an amino acid sequence consisting of amino acid numbers 20 to 467 of the amino acid sequence of SEQ ID NO: 121 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 125, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 467 of the amino acid sequence of SEQ ID NO: 121 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 127, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 467 of the amino acid sequence of SEQ ID NO: 121 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 129, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 467 of the amino acid sequence of SEQ ID NO: 123 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 125, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 467 of the amino acid sequence of SEQ ID NO: 123 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 127, and an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 467 of the amino acid sequence of SEQ ID NO: 123 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 129.

[0136]

Further examples of the antibody of the present invention can include a human antibody. The anti-ALK2 human antibody means a human antibody produced from only human chromosome-derived antibody gene sequences. The anti-ALK2 human antibody can be obtained by a method using human antibody-producing mice carrying human chromosome fragments that comprise human antibody heavy and light chain genes (see e.g., Tomizuka, K.

et al., *Nature Genetics* (1997), 16, p. 133-143; Kuroiwa, Y. et al., *Nuc. Acids Res.* (1998), 26, p. 3447-3448; Yoshida, H. et al., *Animal Cell Technology: Basic and Applied Aspects* vol. 10, p. 69-73 (Kitagawa, Y., 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).

[0137]

Specifically, such a human antibody-producing mouse can be created as a recombinant animal in which the endogenous immunoglobulin heavy and light chain gene loci have been disrupted and instead human immunoglobulin heavy and light chain gene loci are integrated via a vector such as a human artificial chromosome (HAC) vector or a mouse artificial chromosome (MAC) vector, by preparing a knockout animal or a transgenic animal or by crossing these animals.

[0138]

Alternatively, eukaryotic cells may be transformed with cDNAs encoding the heavy and light chains, respectively, of such a human antibody, preferably with vectors comprising the cDNAs, by a gene recombination technique. The transformed cells producing a recombinant human monoclonal antibody are cultured. This antibody can be obtained from the culture supernatant. In this context, for example, eukaryotic cells, preferably mammalian cells such as CHO cells, lymphocytes, or myeloma cells, can be used as hosts.

[0139]

Also, a method for obtaining a phage display-derived human antibody selected from a human antibody library (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) is known.

[0140]

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

to gene analysis to determine DNA sequences encoding the variable regions of the human antibody binding to the antigen. If the DNA sequence of scFv binding to the antigen is determined, an expression vector having this sequence can be prepared and transferred to appropriate hosts, followed by expression to obtain the human antibody (WO92/01047, WO92/20791, WO93/06213, WO93/11236, WO93/19172, WO95/01438, WO95/15388, *Annu. Rev. Immunol.* (1994), 12, p. 433-455; and *Nature Biotechnology* (2005), 23 (9), p. 1105-1116).

[0141]

Antibodies binding to the same epitope as that for the antibody provided by the present invention are also included in the antibodies of the present invention. Examples thereof include antibodies binding to the same epitope as that for the A2-11E antibody, the A2-15A antibody, the A2-25C antibody, and/or the A2-27D antibody.

[0142]

When an antibody binds to or recognizes a partial conformation of an antigen, the epitope for this antibody can be determined by identifying amino acid residues on the antigen adjacent to the antibody by use of X-ray structure analysis. For example, the antibody or a fragment thereof and the antigen or a fragment thereof can be bound to each other, crystallized, and structurally analyzed to identify amino acid residues on the antigen having an interaction distance between the amino acid residue and the antibody. The interaction distance is 8 angstroms or smaller, preferably 6 angstroms or smaller, more preferably 4 angstroms or smaller. One or more amino acid residues having such an interaction distance with the antibody can constitute the epitope (antigenic determinant) for the antibody. When the number of such amino acid residues is two or more, these amino acids may not be adjacent to each other on the primary sequence.

[0143]

A Fab fragment of the chimeric A2-27D antibody and a peptide containing an ECD fragment (amino acid numbers 21 to 123 of SEQ ID NO: 84) of human ALK2 are bound to each other and crystallized under conditions involving 2% (v/v) Tacsimate (pH 7.0), 100 mM HEPES (pH 7.5), and 20% (w/v) polyethylene glycol 3,350 to obtain crystals having a body-

centered monoclinic crystal system with a space group C121 and unit cells of $a = c = 119.39$ angstroms, $b = 37.32$ angstroms, and $\beta = 92.54$. The phase can be determined by a molecular replacement method using three-dimensional structure coordinates thereof (see Example 16).

[0144]

The A2-27D antibody recognizes a partial conformation on human ALK2. In the amino acid sequence (SEQ ID NO: 84) of human ALK2, the amino acid residues having an interaction distance with the A2-27D antibody, i.e., the epitope, is constituted by each of the residues of glutamic acid (Glu) at position 18, glycine (Gly) at position 19, isoleucine (Ile) at position 39, asparagine (Asn) at position 40, aspartic acid (Asp) at position 41, glycine (Gly) at position 42, phenylalanine (Phe) at position 43, histidine (His) at position 44, valine (Val) at position 45, tyrosine (Tyr) at position 46, asparagine (Asn) at position 82, threonine (Thr) at position 84, glutamine (Gln) at position 86, leucine (Leu) at position 87, proline (Pro) at position 88, and threonine (Thr) at position 89. The antibody, an antigen-binding fragment thereof, or a modified form of the antibody or the fragment which binds to this epitope or has an interaction distance between the antibody or the fragment and each of the amino acid residues are also encompassed by the antibody of the present invention, the antigen-binding fragment thereof, or a modified form of the antibody or the fragment.

[0145]

A Fab fragment of the chimeric A2-25C antibody and a peptide containing an ECD fragment (amino acid numbers 21 to 123 of SEQ ID NO: 84) of human ALK2 are bound to each other and crystallized under conditions involving 0.15 M Li_2SO_4 , 0.1 M Na citrate (pH 3.4), 18% (w/v) PEG6,000, and 20% (v/v) ethylene glycol to obtain crystals having an orthorhombic crystal system with a space group $\text{P}2_12_12_1$ and unit cells of $a = 74.49$ angstroms, $b = 128.05$ angstroms, and $c = 147.73$ angstroms. The phase can be determined by a molecular replacement method using three-dimensional structure coordinates thereof (see Example 21).

[0146]

The A2-25C antibody recognizes a partial conformation on human ALK2. In the amino acid sequence (SEQ ID NO: 84) of human ALK2, the amino acid residues having an

interaction distance with the A2-25C antibody, i.e., the epitope, is constituted by each of the residues of glutamic acid (Glu) at position 18, glycine (Gly) at position 19, leucine (Leu) at position 20, isoleucine (Ile) at position 39, asparagine (Asn) at position 40, aspartic acid (Asp) at position 41, glycine (Gly) at position 42, phenylalanine (Phe) at position 43, histidine (His) at position 44, valine (Val) at position 45, tyrosine (Tyr) at position 46, and threonine (Thr) at position 84. The antibody, an antigen-binding fragment thereof, or a modified form of the antibody or the fragment which binds to this epitope or has an interaction distance with these amino acid residues are also encompassed by the antibody of the present invention, the antigen-binding fragment thereof, or a modified form of the antibody or the fragment.

[0147]

The antibody described above can be evaluated for its binding activity to the antigen by, for example, a method described in Example 2, 6, 9, or 10 to select suitable antibodies. The dissociation constant of the antibody is, for example, 1×10^{-6} to 1×10^{-12} M, but is not limited to this range as long as the antibody of the present invention produces the therapeutic or prophylactic effects of interest. The dissociation constant of the antibody for the antigen (ALK2) can be measured using Biacore T200 (GE Healthcare Bio-Sciences Corp.) based on surface plasmon resonance (SPR) as detection principles. For example, the antibody set to an appropriate concentration is reacted as an analyte with the antigen immobilized as a ligand on a solid phase. The association and dissociation therebetween can be measured to obtain an association rate constant k_{a1} , a dissociation rate constant k_{d1} , and a dissociation constant (KD; $KD = k_{d1} / k_{a1}$). The evaluation of binding activity against ALK2 is not limited by use of Biacore T200 and may be conducted using, for example, an instrument based on surface plasmon resonance (SPR) as detection principles, KinExA (Sapidyne Instruments Inc.) based on kinetic exclusion assay as detection principles, BLItz system (Pall Corp.) based on bio-layer interferometry as detection principles, or ELISA (enzyme-linked immunosorbent assay).

[0148]

One example of another index for comparing the properties of antibodies can include the stability of the antibodies. Differential scanning calorimetry (DSC) is a method that can rapidly and accurately measure a transition midpoint (T_m), which serves as a good index for

the relative structural stability of proteins. T_m values can be measured using DSC and compared to determine difference in thermal stability. The preservation stability of an antibody is known to correlate with the thermal stability of the antibody to some extent (Lori Burton, et al., *Pharmaceutical Development and Technology* (2007) 12, p. 265-273). A suitable antibody can be selected with its thermal stability as the index. Examples of other indexes for selecting the antibody can include high yields in appropriate host cells and low aggregation in an aqueous solution. For example, an antibody having the highest yield does not always exhibit the highest thermal stability. Therefore, it is necessary to select an antibody most suitable for administration to humans by comprehensive judgment based on the indexes mentioned above.

[0149]

A method for obtaining a single-chain immunoglobulin by linking the full-length sequences of antibody heavy and light chains using an appropriate linker is also known (Lee, H-S, et al., *Molecular Immunology* (1999) 36, p. 61-71; and Shirrmann, T. et al., *mAbs* (2010), 2, (1) p. 1-4). Such single-chain immunoglobulins can be dimerized to retain a structure and activity similar to those of antibodies which are originally tetramers. Alternatively, the antibody of the present invention may be an antibody that has a single heavy chain variable region and lacks a light chain sequence. Such an antibody, called single-domain antibody (sdAb) or nanobody, has actually been observed in camels or llamas and has been reported to maintain the ability to bind to an antigen (Muyldemans S. et al., *Protein Eng.* (1994) 7 (9), 1129-35; and Hamers-Casterman C. et al., *Nature* (1993) 363 (6428) 446-8). These antibodies may also be interpreted as an antigen-binding fragment of the antibody according to the present invention.

[0150]

The antibody-dependent cellular cytotoxic activity of the antibody of the present invention can be enhanced by adjusting the modification of the sugar chain bound with the antibody. For example, methods described in WO99/54342, WO2000/61739, and WO2002/31140 are known as such a technique of adjusting the sugar chain modification of the antibody, though this technique is not limited thereto.

[0151]

In the case of preparing an antibody by temporarily isolating the antibody gene and then transferring the gene to an appropriate host, the appropriate host can be used in combination with an expression vector.

[0152]

Specific examples of the antibody gene can include a gene (or a polynucleotide) encoding a heavy chain sequence and a gene (or a polynucleotide) encoding a light chain sequence of the antibody described herein, and a combination of these genes (or polynucleotides).

[0153]

Specific examples of the polynucleotide encoding the antibody or an antibody constituent (heavy or light chain) are as follows.

[0154]

- (1) A polynucleotide comprising the following polynucleotides (a) and/or (b):
 - (a) a polynucleotide selected from the group consisting of the following nucleotide sequences:
 - a1) a nucleotide sequence consisting of nucleotide numbers 58 to 426 of the nucleotide sequence of SEQ ID NO: 27;
 - a2) a nucleotide sequence consisting of nucleotide numbers 58 to 426 of the nucleotide sequence of SEQ ID NO: 29;
 - a3) a nucleotide sequence consisting of nucleotide numbers 58 to 426 of the nucleotide sequence of SEQ ID NO: 104;
 - a4) a nucleotide sequence having at least 95% identity to any of the nucleotide sequences a1) to a3);
 - a5) a nucleotide sequence having at least 99% identity to any of the nucleotide sequences a1) to a3);
 - a6) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any of the nucleotide sequences a1) to a3); and

a7) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in the nucleotide sequence a1) or a2); and/or

(b) a polynucleotide selected from the group consisting of the following nucleotide sequences:

b1) a nucleotide sequence consisting of nucleotide numbers 86 to 424 of the nucleotide sequence of SEQ ID NO: 31;

b2) a nucleotide sequence consisting of nucleotide numbers 86 to 424 of the nucleotide sequence of SEQ ID NO: 33;

b3) a nucleotide sequence consisting of nucleotide numbers 86 to 424 of the nucleotide sequence of SEQ ID NO: 35;

b4) a nucleotide sequence consisting of nucleotide numbers 86 to 424 of the nucleotide sequence of SEQ ID NO: 37;

b5) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b4);

b6) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b4);

b7) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences b1) to b4); and

b8) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences b1) to b4).

[0155]

(2) A polynucleotide comprising the following polynucleotides (a) and/or (b):

(a) a polynucleotide selected from the group consisting of the following nucleotide sequences:

a1) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 39;

a2) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 41;

- a3) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 43;
- a4) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 45;
- a5) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 47;
- a6) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 106;
- a7) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 108;
- a8) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences a1) to a7);
- a9) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences a1) to a7);
- a10) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences a1) to a7); and
- a11) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences a1) to a7); and/or
- (b) a polynucleotide selected from the group consisting of the following nucleotide sequences:
 - b1) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 49;
 - b2) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 51;
 - b3) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 53;
 - b4) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 55;

- b5) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 57;
- b6) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b5);
- b7) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b5);
- b8) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences b1) to b5); and
- b9) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences b1) to b5).

[0156]

(3) A polynucleotide comprising the following polynucleotides (a) and/or (b):

(a) a polynucleotide selected from the group consisting of the following nucleotide sequences:

- a1) a nucleotide sequence consisting of nucleotide numbers 58 to 411 of the nucleotide sequence of SEQ ID NO: 110;
- a2) a nucleotide sequence consisting of nucleotide numbers 58 to 411 of the nucleotide sequence of SEQ ID NO: 112;
- a3) a nucleotide sequence having at least 95% identity to the nucleotide sequence a1) or a2);
- a4) a nucleotide sequence having at least 99% identity to the nucleotide sequence a1) or a2);
- a5) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to the nucleotide sequence a1) or a2); and
- a6) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in the nucleotide sequence a1) or a2); and/or

(b) a polynucleotide selected from the group consisting of the following nucleotide sequences:

- b1) a nucleotide sequence consisting of nucleotide numbers 61 to 384 of the nucleotide sequence of SEQ ID NO: 114;

- b2) a nucleotide sequence consisting of nucleotide numbers 61 to 384 of the nucleotide sequence of SEQ ID NO: 116;
- b3) a nucleotide sequence consisting of nucleotide numbers 61 to 384 of the nucleotide sequence of SEQ ID NO: 118;
- b4) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b3);
- b5) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b3);
- b6) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences b1) to b3); and
- b7) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences b1) to b3).

[0157]

(4) A polynucleotide comprising the following polynucleotides (a) and/or (b):

- (a) a polynucleotide selected from the group consisting of the following nucleotide sequences:
 - a1) a nucleotide sequence consisting of nucleotide numbers 58 to 411 of the nucleotide sequence of SEQ ID NO: 120;
 - a2) a nucleotide sequence consisting of nucleotide numbers 58 to 411 of the nucleotide sequence of SEQ ID NO: 122;
 - a3) a nucleotide sequence having at least 95% identity to the nucleotide sequence a1) or a2);
 - a4) a nucleotide sequence having at least 99% identity to the nucleotide sequence a1) or a2);
 - a5) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to the nucleotide sequence a1) or a2); and
 - a6) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in the nucleotide sequence a1) or a2); and/or
- (b) a polynucleotide selected from the group consisting of the following nucleotide sequences:

- b1) a nucleotide sequence consisting of nucleotide numbers 61 to 387 of the nucleotide sequence of SEQ ID NO: 124;
- b2) a nucleotide sequence consisting of nucleotide numbers 61 to 387 of the nucleotide sequence of SEQ ID NO: 126;
- b3) a nucleotide sequence consisting of nucleotide numbers 61 to 387 of the nucleotide sequence of SEQ ID NO: 128;
- b4) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b3);
- b5) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b3);
- b6) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences b1) to b3); and
- b7) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences b1) to b3).

[0158]

For the transformation of host cells, a heavy chain sequence gene (or polynucleotide) and a light chain sequence gene (or polynucleotide) may be inserted in a same expression vector or may be inserted in separate expression vectors. In the case of using host eukaryotic cells, animal cells, plant cells, or eukaryotic microorganisms can be used. Examples of the animal cells can include mammalian cells, for example, monkey COS cells (Gluzman, Y., Cell (1981) 23, p. 175-182, ATCC CRL-1650), mouse fibroblast NIH3T3 (ATCC No. CRL-1658), and dihydrofolate reductase-deficient cell lines (Urlaub, G. and Chasin, L.A., Proc. Natl. Acad. Sci. U.S.A. (1980) 77, p. 4126-4220) of Chinese hamster ovary cells (CHO cells, ATCC CCL-61). In the case of using prokaryotic cells, examples thereof can include *E. coli* and *Bacillus subtilis*. The antibody gene of interest is transferred to these cells by transformation, and the transformed cells are cultured *in vitro* to obtain antibodies. Such culture methods may differ in yield depending on the sequences of the antibodies. An antibody that is easy to produce as

a drug can be selected with its yield as an index from among antibodies having equivalent binding activity.

[0159]

Examples of the isotype of the antibody of the present invention can include, but are not limited to, IgG (IgG1, IgG2, IgG3, and IgG4), IgM, IgA (IgA1 and IgA2), IgD, and IgE. Preferred examples of the isotype can include IgG and IgM, more preferably IgG1, IgG2, and IgG4.

[0160]

In the case of using IgG1 as an isotype of the antibody of the present invention, the effector functions can be adjusted by substitution of a part of amino acid residues in constant regions (see WO88/07089, WO94/28027, and WO94/29351). Examples of such a variant of IgG1 include IgG1 LALA (IgG1-L234A,L235A) and IgG1 LAGA (IgG1-L235A,G237A). IgG1 LALA is preferred.

[0161]

In the case of using IgG4 as an isotype of the antibody of the present invention, splitting unique to IgG4 can be suppressed with extended half-life by substitution of a part of amino acid residues in constant regions (see Molecular Immunology, 30, 1 105-108 (1993)). Examples of such a mutant of IgG4 include IgG4 pro (IgG4-S241P).

[0162]

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

A fragment of the antibody can be obtained by treating the antibody with a proteolytic enzyme such as papain or pepsin or by expressing a genetically engineered antibody gene in appropriate cultured cells. Among such antibody fragments, a fragment that maintains the whole or a portion of the functions possessed by the full-length molecule of the antibody can be referred to as an antigen-binding fragment of the antibody. Examples of the functions of the antibody can generally include antigen binding activity, activity of inhibiting the activity of the antigen, activity of enhancing the activity of the antigen, antibody-dependent cellular cytotoxic activity, complement-dependent cytotoxic activity, and complement-dependent

cellular cytotoxic activity. The function possessed by the antigen-binding fragment of the antibody according to the present invention is an activity of binding to ALK2 and is preferably an activity of inhibiting the activity of ALK2, more preferably an activity of inhibiting ALK2-mediated BMP signal transduction, most preferably an activity of suppressing ectopic ossification and/or bone dysplasia.

[0163]

Examples of the fragment of the antibody can include Fab, F(ab')₂, Fv, single-chain Fv (scFv) comprising heavy and light chain Fvs linked via an appropriate linker, diabody or diabodies, linear antibodies, and multispecific antibodies formed from antibody fragments. The fragment of the antibody also includes Fab', which is a monovalent fragment of antibody variable regions obtained by the treatment of F(ab')₂ under reducing conditions.

[0164]

The antibody of the present invention may be a multispecific antibody having specificity for at least two different types of antigens. Such a molecule usually binds to two types of antigens (i.e., a bispecific antibody). The "multispecific antibody" according to the present invention encompasses an antibody having specificity for more types (e.g., 3 types) of antigens.

[0165]

The multispecific antibody of the present invention may be an antibody consisting of a full length, or may be a fragment of such an antibody (e.g., a bispecific antibody). The bispecific antibody may be prepared by linking the heavy and light chains (HL pairs) of two types of antibodies, or may be prepared by fusing hybridomas producing different monoclonal antibodies to prepare bispecific antibody-producing fusion cells (Millstein et al., Nature (1983) 305, p. 537-539).

[0166]

The antibody of the present invention may be single-chain Fv (also referred to as scFv). The single-chain Fv is obtained by linking the heavy and light chain variable regions of the antibody via a polypeptide linker (Pluckthun, The Pharmacology of Monoclonal Antibodies, 113 (Rosenberg and Moore ed., Springer Verlag, New York, p. 269-315 (1994); and Nature

Biotechnology (2005), 23, p. 1126-1136). Also, a bscFv fragment prepared by linking two scFvs via a polypeptide linker can also be used as a bispecific antibody.

[0167]

The method for preparing the single-chain Fv is 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 chain variable region and the light chain variable region are linked via a linker that prevents these regions from forming a conjugate, preferably a polypeptide linker (Huston, J.S. et al., Proc. Natl. Acad. Sci. U.S.A. (1988), 85, p. 5879-5883). The heavy chain variable region and the light chain variable region in the scFv may be derived from the same antibody or may be derived from different antibodies. For example, an arbitrary single-chain peptide consisting of 12 to 19 residues is used as the polypeptide linker that links these variable regions.

[0168]

In order to obtain DNA encoding the scFv, each DNA portion encoding the whole or desired amino acid sequence in the sequences of DNA encoding the heavy chain or heavy chain variable region of the antibody and DNA encoding the light chain or light chain variable region thereof, is used as a template and amplified by PCR using a primer pair flanking both ends of the template. Subsequently, DNA encoding the polypeptide linker moiety is further amplified in combination with a primer pair defined so that the polypeptide linker moiety can be linked at its ends to the heavy and light chain DNAs, respectively.

[0169]

Once the DNA encoding the scFv is prepared, an expression vector comprising the DNA and a host transformed with the expression vector can be obtained by routine methods. In addition, the host can be used to obtain the scFv according to a routine method. These antibody fragments can be produced by a host in the same way as above by obtaining and expressing the gene.

[0170]

The antibody of the present invention may have enhanced affinity for the antigen by multimerization. Antibodies of the same type may be multimerized, or a plurality of antibodies recognizing a plurality of epitopes, respectively, of the same antigen may be

multimerized. Examples of a method for multimerizing these antibodies can include the binding of two scFvs to an IgG CH3 domain, the binding thereof to streptavidin, and the introduction of a helix-turn-helix motif.

[0171]

The antibody of the present invention may be a polyclonal antibody which is a mixture of plural types of anti-ALK2 antibodies differing in amino acid sequence. One example of the polyclonal antibody can include a mixture of plural types of antibodies differing in CDRs. An antibody obtained by culturing a mixture of cells producing different antibodies, followed by purification from the cultures can be used as such a polyclonal antibody (see WO2004/061104).

[0172]

The antibody of the present invention may be an antibody having 80% to 99% identity as compared with the heavy and/or light chains of the antibody. In this context, the term "identity" has general definition used in the art. The % identity refers to the percentage of the number of identical amino acids relative to the total number of amino acids (including gaps) when two amino acid sequences are aligned so as to give the largest consistency of amino acids. Antibodies that have an ability to bind to the antigen and an inhibitory effect on BMP signal transduction at analogous levels to the antibodies described above can be selected by combining sequences that exhibit high identity to the amino acid sequences of the heavy and light chains. Such identity is generally 80% or higher identity, preferably 90% or higher identity, more preferably 95% or higher identity, most preferably 99% or higher identity. Alternatively, antibodies that have various effects equivalent to the antibodies described above may be selected by combining amino acid sequences that comprise a substitution(s), a deletion(s), and/or an addition(s) of one to several amino acid residues in the amino acid sequences of the heavy and/or light chains. The number of amino acid residues to be substituted, deleted, and/or added is generally 10 or less amino acid residues, preferably 5 or 6 or less amino acid residues, more preferably 2 or 3 or less amino acid residues, most preferably 1 amino acid residue.

[0173]

The heavy chain of an antibody produced by cultured mammalian cells is known to lack a carboxyl-terminal lysine residue (Journal of Chromatography A, 705: 129-134 (1995)). Also, the heavy chain of such an antibody is known to lack two carboxyl-terminal amino acid residues (glycine and lysine) and instead have an amidated proline residue at the carboxy terminus (Analytical Biochemistry, 360: 75-83 (2007)).

[0174]

An amino-terminal glutamine or glutamic acid residue in the heavy or light chain of an antibody is known to be modified by pyroglutamylation during preparation of the antibody, and the antibody of the present invention may have such a modification (WO2013/147153).

[0175]

Such deletion in the heavy chain sequence or modification in the heavy or light chain sequence does not influence the ability of the antibody to bind to the antigen and its effector functions (complement activation, antibody-dependent cytotoxic effects, etc.).

[0176]

Thus, the present invention also encompasses an antibody that has received the deletion or the modification. Examples thereof can include a deletion variant derived from a heavy chain by the deletion of 1 or 2 amino acids at its carboxyl terminus, an amidated form of the deletion variant (e.g., a heavy chain having an amidated proline residue at the carboxyl-terminal site), and an antibody having a pyroglutamylated amino-terminal amino acid residue in a heavy or light chain thereof. However, the deletion variant at the carboxyl terminus of the antibody heavy chain according to the present invention is not limited to the types described above as long as the deletion variant maintains the ability to bind to the antigen and the effector functions. Two heavy chains constituting the antibody according to the present invention may be heavy chains of any one type selected from the group consisting of the full-length heavy chain and the deletion variants described above, or may be a combination of heavy chains of any two types selected therefrom. The quantitative ratio of each deletion variant may be influenced by the type of cultured mammalian cells producing the antibody according to the present invention, and culture conditions. Examples of such a case can

include the deletion of one carboxyl-terminal amino acid residue each in both the two heavy chains as main components of the antibody according to the present invention.

[0177]

The identity between two types of amino acid sequences can be determined using the default parameters of Blast algorithm version 2.2.2 (Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25: 3389-3402). The Blast algorithm is also available by access to www.ncbi.nlm.nih.gov/blast on the Internet. Two types of percentage values, Identity (or Identities) and Positivity (or Positivities), are calculated according to the Blast algorithm. The former is a value that indicates identical amino acid residues between two types of amino acid sequences between which the identity should be determined. The latter is a numerical value determined by also taking into consideration similar amino acid residues in terms of their chemical structures. Herein, the value of identity is defined as the value of "Identity" when amino acid residues are identical between the amino acid sequences.

[0178]

An antibody conjugated with any of various molecules such as polyethylene glycol (PEG) can also be used as a modified form of the antibody.

[0179]

The antibody of the present invention may further be any of conjugates formed by these antibodies with other drugs (immunoconjugates). Examples of such an antibody can include the antibody conjugated with a radioactive material or a compound having a pharmacological effect (*Nature Biotechnology* (2005) 23, p. 1137-1146).

[0180]

The obtained antibody can be purified until homogeneous. A protein separation and purification method usually used can be used for the separation and purification of the antibody. The antibody can be separated and purified by appropriately selected or combined approaches, for example, column chromatography, filtration through a filter, ultrafiltration, salting-out, dialysis, preparative polyacrylamide gel electrophoresis, and/or 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 and purification method is not limited thereto.

[0181]

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

[0182]

These chromatography approaches can be carried out using liquid chromatography such as HPLC or FPLC.

[0183]

Examples of the column for use in the affinity chromatography can include protein A columns and protein G columns.

[0184]

Examples of the protein A columns can include Hyper D, POROS, and Sepharose F.F. (GE Healthcare Bio-Sciences Corp.).

[0185]

Also, the antibody may be purified by exploiting its binding activity to the antigen using an antigen-immobilized carrier.

[0186]

5. Drug comprising anti-ALK2 antibody

An antibody inhibiting the biological activity of ALK2 can be obtained from among anti-ALK2 antibodies obtained by the methods described above in the preceding paragraph "4. Production of anti-ALK2 antibody". Such an antibody inhibiting the biological activity of ALK2 inhibits the biological activity of ALK2, i.e., ALK2-mediated BMP signal transduction, *in vivo* and as such, can be pharmaceutically used as a therapeutic and/or prophylactic drug for ectopic ossification and/or bone dysplasia or a therapeutic and/or prophylactic drug for anemia.

[0187]

Examples of the disease that can be treated and/or prevented with the anti-ALK2 antibody can include fibrodysplasia ossificans progressiva (FOP), progressive osseous heteroplasia (POH), traumatic ectopic ossification, ectopic ossification after implant arthroplasty, spondyloarthritis (SpA), ankylosing spondylitis (AS), anemia, diffuse intrinsic pontine glioma (DIPG), and thinning hair. The disease is preferably fibrodysplasia ossificans progressiva (FOP), progressive osseous heteroplasia (POH), traumatic ectopic ossification, or ectopic ossification after implant arthroplasty, more preferably fibrodysplasia ossificans progressiva (FOP), though the disease is not limited thereto as long as the disease is caused by ALK2-mediated BMP signal transduction. In FOP patients, finger or toe fusion or deformity, cervical fusion or deformity, or the like is also found, and hearing loss is also manifested. These conditions are also included in the disease caused by ALK2-mediated BMP signal transduction.

[0188]

Activating mutations in ALK2 have been confirmed in all FOP patients, and 10 or more types of mutations have been reported so far. All of these mutations have been found to be amino acid mutations (missense mutations) present in the intracellular region of the ALK2 protein and do not cause any change in the amino acid sequence of the extracellular region. The antibody of the present invention binds to the extracellular region of ALK2 and as such, can be used as a therapeutic and/or prophylactic drug for every FOP patient, irrespective of the types of mutations.

[0189]

The treatment of FOP means cure of FOP symptoms, amelioration of the symptoms, mitigation of the symptoms, or suppression of progression of the symptoms.

[0190]

The prevention of FOP means circumvention or suppression of onset of flare-up or ectopic ossification.

[0191]

The antibody of the present invention also binds not only to mutated ALK2 but to wild-type ALK2 and inhibits downstream signals. Therefore, the antibody of the present invention

can also be used as a therapeutic and/or prophylactic drug for non-hereditary ectopic ossification different from FOP. Examples of the non-hereditary ectopic ossification include ectopic ossification after brain contusion, ectopic ossification after spinal cord injury, ectopic ossification after burn injury, and ectopic ossification after implant arthroplasty. The involvement of neuropeptides, fat cells, and immune system cells such as macrophages has been reported as a cause for the non-hereditary ectopic ossification (see *J. Cell. Biochem.*, 112, 10 (2011); *J. Cell. Biochem.*, 112, 10 (2011); and *J. Pathol.*, 236, 2 (2015)). It has been suggested that a similar mechanism is also involved in flare-up in FOP patients (see *Hum. Pathol.*, 32, 8 (2001); and *Histol. Histopathol.*, 29, 10 (2014)). Thus, the antibody of the present invention can be used as a therapeutic and/or prophylactic drug not only for ectopic ossification in FOP but for non-hereditary ectopic ossification.

[0192]

Hepcidin gene is known as a target gene whose transcription is positively regulated by the BMP-ALK signal transduction pathway in anemia (see *Blood*, 118, 15 (2011)). Hepcidin is a peptide hormone mainly produced in the liver, and regulates the degradation of a transporter, called ferroportin, which is expressed in the gastrointestinal tract and involved in iron absorption (see *Science*, 306, 5704 (2004)). A potential therapeutic drug for anemia has been reported because the functional inhibition of hepcidin or the suppression of its expression level leads to the promotion of iron uptake from the gastrointestinal tract via increase in the expression level of ferroportin (see *Pharmacol Ther*, 146 (2015)). Thus, the antibody of the present invention can also be used as a therapeutic and/or prophylactic drug for iron-deficiency anemia by increasing iron absorption via the suppression of hepcidin expression in the liver.

[0193]

Alternatively, the antibody of the present invention may be used as a therapeutic and/or prophylactic drug for diffuse intrinsic pontine glioma (DIPG). DIPG is diffuse (infiltrative) astrocytoma that is found mainly in the pons of the brain stem and reportedly accounts for approximately 75 to 80% of pediatric brain stem tumors. There is a report stating that fewer than about 10% of children with DIPG survive for more than 2 years, because the brain stem

regulates essential functions such as respiration (Khuong-Quang D-A et al., Acta Neuropathol 124: 439-447, 2012). The antibody of the present invention can also be used as a therapeutic and/or prophylactic drug for DIPG.

[0194]

Examples of the anti-ALK2 antibody as these drugs can include chimeric antibodies and humanized antibodies prepared by the methods described in "4. Production of anti-ALK2 antibody" from the A2-15A antibody, the A2-27D antibody, the A2-11E antibody, or the A2-25C antibody. A chimeric antibody, a humanized antibody and a human antibody binding to the same epitope as that for the A2-15A antibody, the A2-27D antibody, the A2-11E antibody, and/or the A2-25C antibody may also be used as the drugs.

[0195]

The biological activity of ALK2 (BMP signal inhibitory activity) of the anti-ALK2 antibody can be confirmed *in vitro*, for example, by luciferase assay using reporter plasmids having an insert of a BMP-responsive sequence, SMAD1/5/8 phosphorylation, expression analysis of BMP target genes, or measurement of alkaline phosphatase activity in mouse myoblasts C2C12 induced to differentiate into osteoblasts by stimulation with a BMP ligand.

[0196]

The therapeutic or prophylactic effects of the anti-ALK2 antibody on ectopic ossification or bone dysplasia can be confirmed *in vivo* using laboratory animals, for example, by subcutaneously or intravenously administering the anti-ALK2 antibody to ectopic ossification-induced models with BMP ligand-containing pellets transplanted to mouse muscle, or FOP mouse models harboring mutated ALK2, and analyzing ectopic bone formation.

[0197]

The anti-ALK2 antibody thus obtained is useful as a drug, particularly, a pharmaceutical composition aimed at treating or preventing ectopic ossification such as fibrodysplasia ossificans progressiva (FOP).

[0198]

As one example, the anti-ALK2 antibody can be administered alone or in combination with at least one additional therapeutic drug for ectopic ossification in the treatment or

prevention of ectopic ossification. As another example, the anti-ALK2 antibody can be administered in combination with a therapeutically effective amount of a therapeutic drug. Examples of the additional therapeutic drug for ectopic ossification that can be administered in combination with the anti-ALK2 antibody can include, but are not limited to, anti-inflammatory drugs, steroids, bisphosphonates, muscle relaxants, and retinoic acid receptor (RAR) γ agonists.

[0199]

Examples of the anti-inflammatory drug can include aspirin, diclofenac, indomethacin, ibuprofen, ketoprofen, naproxen, piroxicam, rofecoxib, celecoxib, azathioprine, penicillamine, methotrexate, sulfasalazine, leflunomide, infliximab, and etanercept. Indomethacin, ibuprofen, piroxicam, or celecoxib is preferred.

[0200]

Examples of the steroid can include prednisolone, beclomethasone, betamethasone, fluticasone, dexamethasone, and hydrocortisone. Prednisolone is preferred.

[0201]

Examples of the bisphosphonate can include alendronate, cimadronate, clodronate, etidronate, ibandronate, incadronate, minodronate, neridronate, olpadronate, pamidronate, piridronate, risedronate, tiludronate, and zoledronate. Pamidronate or zoledronate is preferred.

[0202]

Examples of the muscle relaxant can include cyclobenzaprine, metaxalone, and baclofen. Baclofen is preferred.

[0203]

Examples of the retinoic acid receptor γ agonist can include palovarotene.

[0204]

Depending on the condition of ectopic ossification or the intended degree of treatment and/or prevention, two or three or more additional therapeutic drugs can be administered, and these additional therapeutic drugs can be included in the same preparation and thereby administered at the same time. The additional therapeutic drug and the anti-ALK2 antibody

can also be included in the same preparation and thereby administered at the same time. Also, the anti-ALK2 antibody and the additional therapeutic drug can be included in separate preparations and administered at the same time. Alternatively, the additional agent and the anti-ALK2 antibody may be separately administered one after another. Specifically, a therapeutic drug comprising the anti-ALK2 antibody or the antigen-binding fragment of the antibody as an active ingredient may be administered after administration of the additional therapeutic drug, or the additional therapeutic drug may be administered after administration of the therapeutic drug containing the anti-ALK2 antibody or the antigen-binding fragment of the antibody as an active ingredient. For administration in gene therapy, a gene for a protein serving as a therapeutic drug for ectopic ossification and the gene for the anti-ALK2 antibody can be inserted at a site downstream of separate promoter regions or the same promoter region and can be introduced to separate vectors or the same vector.

[0205]

The anti-ALK2 antibody or the fragment thereof can be conjugated with a therapeutic drug for ectopic ossification to produce a targeted drug conjugate described in M.C. Garnet "Targeted drug conjugates: principles and progress", *Advanced Drug Delivery Reviews*, (2001) 53, 171-216. For this purpose, an antibody molecule as well as any antibody fragment is applicable unless their ALK2-recognizing properties are completely deleted. Examples of the antibody fragment can include fragments such as Fab, F(ab')₂, and Fv. In the present invention as well, the antibody and the fragment can be used. The conjugation manner of the anti-ALK2 antibody or the fragment of the antibody with the therapeutic drug for FOP can take various forms described in, for example, M.C. Garnet "Targeted drug conjugates: principles and progress", *Advanced Drug Delivery Reviews*, (2001) 53, 171-216, G.T. Hermanson "Bioconjugate Techniques" Academic Press, California (1996), Putnam and J. Kopecek "Polymer Conjugates with Anticancer Activity" *Advances in Polymer Science* (1995) 122, 55-123. Specific examples thereof can include a manner in which the anti-ALK2 antibody is chemically conjugated with the therapeutic drug for ectopic ossification either directly or via a spacer such as an oligopeptide, and a manner in which the anti-ALK2 antibody is conjugated with the therapeutic drug for ectopic ossification via an appropriate

drug carrier. Examples of the drug carrier can include liposomes and water-soluble polymers. Examples of such a manner via the drug carrier can more specifically include a manner in which the therapeutic drug for ectopic ossification is encapsulated in a liposome and the liposome is conjugated with the antibody, and a manner in which the therapeutic drug for ectopic ossification is chemically conjugated with a water-soluble polymer (compound having a molecular weight on the order of 1000 to 100,000) either directly or via a spacer such as an oligopeptide and the water-soluble polymer is conjugated with the antibody. The conjugation of the antibody (or the fragment) with the therapeutic drug for ectopic ossification or the drug carrier (e.g., a liposome or a water-soluble polymer) can be carried out by a method well known to those skilled in the art, such as a method described in G.T. Hermanson "Bioconjugate Techniques" Academic Press, California (1996), and Putnam and J. Kopecek "Polymer Conjugates with Anticancer Activity" Advances in Polymer Science (1995) 122, 55-123. The encapsulation of the therapeutic drug for ectopic ossification in the liposome can be carried out by a method well known to those skilled in the art, such as a method described in, for example, D.D. Lasic "Liposomes: From Physics to Applications", Elsevier Science Publishers B.V., Amsterdam (1993). The conjugation of the therapeutic drug for ectopic ossification with the water-soluble polymer can be carried out by a method well known to those skilled in the art, such as a method described in D. Putnam and J Kopecek "Polymer Conjugates with Anticancer Activity" Advances in Polymer Science (1995) 122, 55-123. The conjugate of the antibody (or the fragment) with the protein as a therapeutic drug for ectopic ossification (or a fragment thereof) can be prepared by any of the methods described above or a genetic engineering method well known to those skilled in the art.

[0206]

The present invention also provides a pharmaceutical composition comprising a therapeutically and/or prophylactically effective amount of the anti-ALK2 antibody and a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative, and/or auxiliary agent.

[0207]

The present invention also provides a pharmaceutical composition comprising a therapeutically and/or prophylactically effective amount of the anti-ALK2 antibody, a therapeutically and/or prophylactically effective amount of at least one therapeutic drug for ectopic ossification, and a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative, and/or auxiliary agent.

[0208]

It is preferred that the pharmaceutically acceptable material used in the pharmaceutical composition of the present invention should be nontoxic to a recipient of the pharmaceutical composition, preferably in terms of a dose or an administered concentration.

[0209]

The pharmaceutical composition of the present invention can comprise pharmaceutical materials for changing or maintaining pH, osmotic pressure, viscosity, transparency, color, tonicity, sterility, stability, solubility, sustained release, absorbability, or permeability. Examples of the pharmaceutical materials can include, but are 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, or borate buffers, sodium bicarbonate, and Tris-HCl solutions; fillers such as mannitol and glycine; chelating agents such as ethylenediaminetetraacetic acid (EDTA); complexing agents such as caffeine, polyvinylpyrrolidone, β -cyclodextrin, and hydroxypropyl- β -cyclodextrin; bulking agents such as glucose, mannose, and dextrin; other carbohydrates such as monosaccharides and disaccharides; coloring agents; corrigents; diluents; emulsifiers; hydrophilic polymers such as polyvinylpyrrolidone; 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 sorbitan ester, polysorbates such as polysorbate 20 and polysorbate 80, triton, tromethamine, lecithin, and cholesterol; stability enhancers such as sucrose and sorbitol; elasticity enhancers such as sodium chloride, potassium chloride, mannitol, and sorbitol;

transport agents; excipients; and/or pharmaceutical auxiliary agents. The amount of these pharmaceutical materials added is preferably 0.01 to 100 times, particularly, 0.1 to 10 times relative to the weight of the anti-ALK2 antibody. The suitable components of the pharmaceutical composition in a preparation can be appropriately determined by those skilled in the art according to an applicable disease, an applicable administration route, etc.

[0210]

The excipient or the carrier in the pharmaceutical composition may be liquid or solid. Appropriate excipients or carriers may be injectable water, physiological saline, artificial cerebrospinal fluids, or other materials usually used in parenteral administration. Neutral physiological saline or physiological saline containing serum albumin may be used as a carrier. The pharmaceutical composition can contain a Tris buffer of pH 7.0 to 8.5, an acetate buffer of pH 4.0 to 5.5, or a citrate buffer of pH 3.0 to 6.2. These buffers can also contain sorbitol or other compounds. Examples of the pharmaceutical composition of the present invention can include a pharmaceutical composition comprising the anti-ALK2 antibody, and a pharmaceutical composition comprising the anti-ALK2 antibody and at least one therapeutic drug for ectopic ossification. The pharmaceutical composition of the present invention is prepared in the form of a freeze-dried product or a liquid as a drug having a selected recipe and a necessary purity. The pharmaceutical composition comprising the anti-ALK2 antibody, or the pharmaceutical composition comprising the anti-ALK2 antibody and at least one therapeutic drug for ectopic ossification can also be formed as a freeze-dried product using an appropriate excipient such as sucrose.

[0211]

The pharmaceutical composition of the present invention may be prepared for parenteral administration or may be prepared for absorption in the gastrointestinal tract through an oral route. The recipe and concentration of the preparation can be determined according to an administration method. As the anti-ALK2 antibody contained in the pharmaceutical composition of the present invention has higher affinity for ALK2, i.e., a lower dissociation constant (KD value) for ALK2, the anti-ALK2 antibody can exert efficacy even at a reduced dose to a human. Therefore, the dose of the pharmaceutical composition of the

present invention to a human can also be determined on the basis of this result. The dose for the administration of the human type anti-ALK2 antibody to a human is, for example, approximately 0.1 to 100 mg/kg, which can be administered once or twice or more per 1 to 180 days. However, the dose and the number of doses should generally be determined in consideration of the sex, body weight, and age of a patient, symptoms, severity, adverse reactions, etc., and therefore, are not limited to the dose or usage described above.

[0212]

Non-limiting examples of the form of the pharmaceutical composition of the present invention can include injections including intravenous drips, suppositories, transnasal formulations, sublingual formulations, and transdermal absorption formulations. The administration route is an oral administration route or a parenteral administration route. Non-limiting examples of the parenteral administration route include intravenous, intraarterial, intramuscular, intrarectal, transmucosal, and intradermal routes.

EXAMPLES

[0213]

The present invention will be specifically described hereinafter with Examples; however, the invention is not limited thereto. In the following Examples, unless otherwise specified, operations concerning genetic manipulation were performed in accordance with methods described in "Molecular Cloning" (Sambrook, J., Fritsch, E.F., and Maniatis, T., Cold Spring Harbor Laboratory Press, 1989), or where commercial reagents or kits were used, they were used in accordance with the manuals for such commercial products.

[0214]

<Example 1>

Preparation of rat anti-ALK2 antibodies (11E2, 15A6, 25C11, and 27D11: hereinafter abbreviated as A2-11E, A2-15A, A2-25C, and A2-27D)

1)-1 Preparation of an antigen

Mouse ALK2-His&Fc (Cat. # 50297-M03H) from Sino Biological Inc. was used as mALK2-Fc, which is the antigen.

[0215]

1)-2 Immunization of animals

1 mg/mL of mouse ALK2-His&Fc was mixed with an equal volume of TiterMaxGold (TiterMax, USA) to prepare an emulsion. To two 6-week-old Wister female rats, 100 µg per rat of the antigen was subcutaneously administered with the adjuvant in two divided doses. After 1 to 2 weeks, 40 µg of only the antigen solution was subcutaneously administered, and after 3 days, spleen cells and various lymph glands were aseptically removed as antibody-producing cells, and subjected to the following cell fusion.

[0216]

1)-3 Cell fusion with myeloma cells

The above-described antibody-producing cells were mixed with a myeloma cell line (P3U1 cells) derived from BALB/c mice at a ratio of 5:1 to 10:1, and fused for 3 minutes using 50% polyethylene glycol 1500, and then diluted over 6 minutes. The fused cells were seeded with feeder cells (thymocytes) into ten 96-well plates per rat. The cells were cultured in 15% FBS-containing RPMI1640 medium (containing glutamine, pyruvic acid, penicillin, and streptomycin) containing HAT supplement.

[0217]

1)-4 Selection of Hybridomas

One week after the cell fusion, using each culture supernatant, clones recognizing mouse ALK2-His&Fc used as the antigen were selected by the enzyme-linked immunosorbent assay (ELISA), and clones recognizing only human Fc (Sino Biological Inc.) were excluded. ELISA was performed as follows.

[0218]

First, the antigen diluted to 1 µg/ml with PBS was immobilized onto a 96-well ELISA plate (NUNC, Cat. # 442404) for 2 hours at room temperature or overnight at 4°C. The immobilized solution was removed, and the plate was blocked with a 0.5% skim milk solution dissolved in PBS for 30 minutes at room temperature. Next, the above-described culture supernatant of hybridomas was added, and the plate was left for 1 hour at room temperature. After the plate was washed, an ALP-labeled anti-rat IgG antibody (SBA, Inc.) diluted with

0.5% skim milk to 1:2500 was added, and the plate was further left for 1 hour at room temperature. After the plate was washed, a phenyl phosphate substrate was reacted for 20 minutes at room temperature, and then absorbance at a wavelength of 492 nm was measured.

[0219]

Selected hybridomas were cloned twice or more by limiting dilution. Through the above-described operations, hybridoma cell lines producing monoclonal antibodies A2-11E, A2-15A, A2-25C, and A2-27D were isolated.

[0220]

The results are shown in Figure 1. The results showed that each of the monoclonal antibodies produced by hybridomas A2-11E, A2-15A, A2-25C, and A2-27D recognized mouse ALK2-His&Fc, and did not bind to human Fc.

[0221]

<Example 2>

In vitro evaluation of rat anti-ALK2 antibodies (A2-11E, A2-15A, A2-25C, and A2-27D)

2)-1 Antibody screening by flow cytometry

2)-1-1 Preparation of mouse and human ALK2-expressing cells

HEK293A cells were seeded into a 100-mm dish at 3×10^4 cells/cm², and cultured overnight in 15% FBS-containing DMEM medium under the conditions of 5% CO₂ at 37 °C. On the following day, the HEK293A cells were transfected with each of pcDEF3/mouse ALK2(WT)-EGFP, pcDEF3/human ALK2(WT)-EGFP, pcDEF3/human ALK2 (R206H)-EGFP, and pcDEF3, using Lipofectamine 2000 (Invitrogen), and further cultured overnight. On the following day, 100 µL each of the cell suspensions adjusted to 1×10^6 cells/mL was dispensed into a 1.5-mL microfuge and centrifuged at 500 g for 5 minutes, and then the supernatant was removed. To the cells, 100 µL of purified IgG diluted to 1 µg/mL was added, and the cells were left for 30 minutes at 4 °C. After the cells were washed with PBS three times, 100 µL of Alexa flour 647 Goat anti-Rat IgG (H+L) (Life Technologies) diluted to 1:200 was added, and the cells were further left for 30 minutes at 4 °C. After the cells were washed with PBS three times, fluorescence was detected by a flow cytometer (FACS

Aria II: BD Biosciences). The data was analyzed using BD Diva Software (BD Biosciences), and the intensity of EGFP expressed by the cells and the fluorescence intensity of stained Alexa flour 647 were plotted.

[0222]

The results are shown in Figures 2A to 2D. It was confirmed that each of the monoclonal antibodies produced by hybridomas A2-11E, A2-15A, A2-25C, and A2-27D did not recognize fluorescence protein EGFP-expressing cells (Figure 2A), but specifically recognized mouse ALK2-expressing cells (Figure 2B) as well as wild-type and FOP human ALK2-expressing cells (Figures 2C and 2D, respectively). These results show that the monoclonal antibodies produced by hybridomas A2-11E, A2-15A, A2-25C, and A2-27D are antibodies that bind to the extracellular region of ALK2.

[0223]

2)-2 Antibody screening by immunostaining

2)-2-1 Preparation of human ALK1-, ALK2-, ALK3-, and ALK6-expressing cells

Mouse C2C12 cells were seeded into a 96-well plate (Greiner Bio-One) at 5×10^3 cells/well, and cultured overnight in 15% FBS-containing DMEM medium under the conditions of 5% CO₂ at 37 °C. On the following day, the C2C12 cells were transfected with each of pcDEF3/human ALK1-EGFP, pcDEF3/human ALK2-EGFP, pcDEF3/human ALK3-EGFP, pcDEF3/human ALK6-EGFP, and pcDEF3 as a control, using Lipofectamine 2000 (Invitrogen), and further cultured overnight.

[0224]

2)-2-2 Immunofluorescence staining of human ALK1, ALK2, ALK3, and ALK6

The C2C12 cells were fixed with 10% neutral formalin (Nacalai Tesque, Japan) for 20 minutes at room temperature, and then blocked with 10% goat normal serum for 30 minutes. After the blocking agent was removed, purified IgG diluted to 10 µg/mL with the blocking agent was added, and the cells were left for 1 hour at room temperature. After the cells were washed with PBS three times, Goat Alexa 594-conjugated anti-rat IgG (Invitrogen) diluted to 1:1000 with the blocking agent was added, and the cells were left for 1 hour at room temperature. After the cells were washed with PBS three times, the cells were fixed with

10% neutral formalin for 15 minutes at room temperature, and nuclear-stained with DAPI (LifeTechnologies). Fluorescent signals were analyzed with a fluorescence microscope BZ-9000 (Keyence).

[0225]

The results are shown in Figure 3. It was confirmed that the monoclonal antibodies produced by hybridomas A2-11E, A2-15A, A2-25C, and A2-27D specifically recognized only ALK2-expressing cells, and did not recognize ALK1-, ALK3-, and ALK6-expressing cells. These results show that the monoclonal antibodies produced by hybridomas A2-11E, A2-15A, A2-25C, and A2-27D are antibodies that specifically bind to ALK2.

[0226]

2)-2-3 Preparation of cells expressing ALK2 mutants identified in FOP

Mouse C2C12 cells were seeded into a 96-well plate (Greiner Bio-One) at 0.5×10^3 cells/well, and cultured in 15% FBS-containing DMEM medium overnight under the conditions of 5% CO₂ at 37 °C. On the following day, the C2C12 cells were transfected with each of a wild-type human ALK2 cDNA integrated into pcDEF3; pcDEF3/human ALK2-L196P, pcDEF3/human ALK2-P197_F198delinsL, pcDEF3/human ALK2-R202I, pcDEF3/human ALK2-R206H, pcDEF3/human ALK2-Q207E, pcDEF3/human ALK2-R258S, pcDEF3/human ALK2-G325A, pcDEF3/human ALK2-G328E, pcDEF3/human ALK2-G328R, pcDEF3/human ALK2-G328W, pcDEF3/human ALK2-G356D, and pcDEF3/human ALK2-R375P, which are variants identified in FOP; and pcDEF3 as a control, using Lipofectamine 2000 (Invitrogen), and further cultured overnight.

[0227]

2)-2-4 Immunofluorescence staining of ALK2 mutants identified in FOP

The C2C12 cells were fixed with 10% neutral formalin (Nacalai Tesque, Japan) for 20 minutes at room temperature, and then blocked with 10% goat normal serum for 30 minutes. After the blocking agent was removed, purified IgG diluted to 10 µg/mL with the blocking agent was added, and the cells were left for 1 hour at room temperature. After the cells were washed with PBS three times, Goat Alexa 594-conjugated anti-rat IgG (Invitrogen) diluted to 1:1000 with the blocking agent was added, and the cells were left for 1 hour at room

temperature. After the cells were washed with PBS three times, the cells were fixed with 10% neutral formalin for 15 minutes at room temperature, and nuclear-stained with DAPI (LifeTechnologies). Fluorescent signals were analyzed with a fluorescence microscope BZ-9000 (Keyence).

[0228]

The results are shown in Figure 4. It was confirmed that the monoclonal antibodies produced by hybridomas A2-11E, A2-15A, A2-25C, and A2-27D recognized the cells expressing the 12 types of ALK2 mutants identified in FOP, in addition to the wild-type ALK2-expressing cells. These results show that the monoclonal antibodies produced by hybridomas A2-11E, A2-15A, A2-25C, and A2-27D are antibodies that bind to wild-type ALK2 and each of the ALK2 mutants in FOP.

[0229]

2)-3 Antibody screening by luciferase reporter assay

The ALK2-mediated intracellular signaling inhibitory activity of each of the monoclonal antibodies was analyzed using a BMP-specific luciferase reporter. HEK293A cells were seeded into a 96-well white plate for luciferase assay (Greiner Bio-One) at 1×10^4 cells/well, and cultured overnight in 15% FBS-containing DMEM medium under the conditions of 5% CO₂ at 37 °C. On the following day, the HEK293A cells were transfected with pcDEF3/human ALK2(WT)-V5-His or pcDEF3/human ALK2(R206H)-V5-His, pGL4.26/Id1WT4F-luc (Genes Cells, 7, 949 (2002)), and phRL SV40 (Promega), using Lipofectamine 2000 (Invitrogen). After 2.5 hours, the medium was replaced with fresh OPTI-MEM I (LifeTechnologies), and the cells were further cultured for 1 hour. Thereafter, the medium was replaced with OPTI-MEM I containing the serially diluted monoclonal antibody and 10 ng/mL of BMP7 (Milteneu) or 0.5 ng/mL of BMP9 (Peprotech), and the cells were further cultured overnight. On the following day, the firefly and Renilla luciferase activities were measured with the plate reader GENios (TECAN), using Dual-Glo Luciferase Assay System (Promega).

[0230]

The results are shown in Figure 5. It was confirmed that the monoclonal antibodies produced by hybridomas A2-11E, A2-15A, A2-25C, and A2-27D suppressed the BMP7-induced BMP-specific luciferase activity in a dose-dependent manner, in the HEK293A cells overexpressing wild-type ALK2 and mutant R206H.

[0231]

2)-4 Antibody screening by BMP-induced osteoblast differentiation induction assay

The endogenous ALK2-mediated intracellular signaling inhibitory activity of each of the monoclonal antibodies was analyzed based on the effect upon the BMP-induced activity of inducing the differentiation of C2C12 cells into osteoblasts. C2C12 cells were seeded into a 96-well plate (Greiner Bio-One) at 0.5×10^3 cells/well, and cultured in 15% FBS-containing DMEM medium overnight under the conditions of 5% CO₂ at 37 °C. On the following day, the medium was replaced with OPTI-MEM I containing the serially diluted monoclonal antibody and 200 ng/mL of BMP2 (Corefront Corporation), 200 ng/mL of BMP7 (Mitene), or 20 ng/mL of GDF2/BMP9 (Peprotech), and the cells were further cultured for 3 days. After the medium was removed from the C2C12 cells and the cells were washed with PBS, the cells were treated with 50 µL/well of an ice-cold acetone:ethanol (1:1) solution for 1 minute, and further washed with PBS three times. ALP activity was measured as an index of the differentiation into cells for forming osteoblasts. To measure ALP activity, 100 µL/well of a substrate solution (0.1 M diethanolamine (Sigma-Aldrich)-HCl, pH 10.0, containing 1 mg/mL of 4-nitrophenyl phosphate (Sigma-Aldrich) and 1 mM of MgCl₂) was added, and reacted for 15 to 30 minutes at room temperature on an agitation shaker. The reaction was stopped by the addition of 50 µL of 3M NaOH, and absorbance at a wavelength of 405 nm was measured using the microplate reader Infinite F50 (TECAN).

[0232]

The results are shown in Figures 6 and 7. It was confirmed that the monoclonal antibodies produced by hybridomas A2-11E, A2-15A, A2-25C, and A2-27D barely suppressed the BMP2-induced differentiation of the C2C12 cells into osteoblast-like cells (Figure 6), but suppressed the BMP7- and GDF2/BMP9-induced differentiation of the C2C12 cells into osteoblast-like cells in a dose-dependent manner (Figure 7). These results show

that the monoclonal antibodies produced by hybridomas A2-11E, A2-15A, A2-25C, and A2-27D are antibodies that suppress endogenous ALK2 physiologically expressed by the C2C12 cells.

[0233]

<Example 3>

Nucleotide sequencing of cDNAs encoding variable regions of rat anti-ALK2 antibodies (A2-11E, A2-15A, A2-25C, and A2-27D)

3)-1 Nucleotide sequencing of cDNAs encoding variable regions of A2-11E

3)-1-1 Preparation of total RNA from A2-11E-producing hybridomas

To amplify cDNAs containing variable regions of A2-11E, total RNA was prepared from the A2-11E-producing hybridomas, using TRIzol Reagent (Ambion).

[0234]

3)-1-2 Synthesis of a cDNA (5'-RACE-Ready cDNA)

A cDNA (5'-RACE-Ready cDNA) was synthesized from 1 µg of the total RNA prepared in Example 3)-1-1, using SMARTer RACE cDNA Amplification Kit (Clontech).

[0235]

3)-1-3 Amplification by 5'-RACE PCR and sequencing of a cDNA containing a heavy chain variable region of A2-11E

UPM (Universal Primer A Mix: included in SMARTer RACE cDNA Amplification Kit) and an oligonucleotide having the sequence: 5'-CTCCAGAGTTCCAGGTCACGGTGACTGGC-3' (RG2AR3: SEQ ID NO: 88) were used as primers for PCR amplification of a cDNA containing the variable region of the heavy chain gene of A2-11E. UPM included in SMARTer RACE cDNA Amplification Kit (Clontech) was used, and RG2AR3 was designed from the sequence of a rat heavy chain constant region on a database.

[0236]

A cDNA containing a heavy chain variable region of A2-11E was amplified by 5'-RACE PCR using this combination of primers and the cDNA (5'-RACE-Ready cDNA) synthesized in Example 3)-1-2 as a template. PCR was performed using KOD -Plus-

(TOYOBO, Japan) as polymerase, and using the touch-down PCR program in accordance with the manual of SMARTer RACE cDNA Amplification Kit (Clontech).

[0237]

The cDNA containing the heavy chain variable region amplified by 5'-RACE PCR was purified using MinElute PCR Purification Kit (QIAGEN), and then cloned using Zero Blunt TOPO PCR Cloning Kit (Invitrogen), and the sequence analysis of the nucleotide sequence of the cloned cDNA containing the heavy chain variable region was performed.

[0238]

As sequence primers, the oligonucleotide having the sequence: 5'-CTCCAGAGTTCCAGGTCACGGTGACTGGC-3' (RG2AR3: SEQ ID NO: 88) designed from the sequence of a rat heavy chain constant region on a database and NUP (Nested Universal Primer A: included in SMARTer RACE cDNA Amplification Kit) were used.

[0239]

The sequence analysis was performed using a gene sequence analyzer ("ABI PRISM 3700 DNA Analyzer; Applied Biosystems" or "Applied Biosystems 3730xl Analyzer; Applied Biosystems"), and the sequence reaction was performed using GeneAmp 9700 (Applied Biosystems).

[0240]

The determined nucleotide sequence of the cDNA encoding the heavy chain variable region of A2-11E is shown in SEQ ID NO: 1 in the Sequence Listing, and the amino acid sequence is shown in SEQ ID NO: 2.

[0241]

3)-1-4 Amplification by 5'-RACE PCR and sequencing of a cDNA containing a light chain variable region of A2-11E

UPM (Universal Primer A Mix: included in SMARTer RACE cDNA Amplification Kit) and an oligonucleotide having the sequence: 5'-TCAGTAACACTGTCCAGGACACCATCTC-3' (RKR5; SEQ ID NO: 89) were used as primers for PCR amplification of a cDNA containing the variable region of the light chain gene of A2-11E. UPM included in SMARTer RACE cDNA Amplification Kit (Clontech)

was used, and RKR5 was designed from the sequence of a rat light chain constant region on a database.

[0242]

A cDNA containing a light chain variable region of A2-11E was amplified by 5'-RACE PCR using this combination of primers and the cDNA (5'-RACE-Ready cDNA) synthesized in Example 3)-1-2 as a template. PCR was performed using KOD -Plus- (TOYOBO, Japan) as polymerase, and using the touch-down PCR program in accordance with the manual of SMARTer RACE cDNA Amplification Kit (Clontech).

[0243]

The cDNA containing the light chain variable region amplified by 5'-RACE PCR was purified using MinElute PCR Purification Kit (QIAGEN), and then cloned using Zero Blunt TOPO PCR Cloning Kit (Invitrogen), and the sequence analysis of the nucleotide sequence of the cloned cDNA containing the light chain variable region was performed.

[0244]

As a sequence primer, the following sequence was designed from the sequence of a rat light chain constant region on a database.

[0245]

The oligonucleotide having the designed sequence: 5'-TCAGTAACACTGTCCAGGACACCATCTC-3' (RKR5; SEQ ID NO: 89) and NUP (Nested Universal Primer A: included in SMARTer RACE cDNA Amplification Kit) were used.

[0246]

The sequence analysis was performed using a gene sequence analyzer ("ABI PRISM 3700 DNA Analyzer; Applied Biosystems" or "Applied Biosystems 3730xl Analyzer; Applied Biosystems"), and the sequence reaction was performed using GeneAmp 9700 (Applied Biosystems).

[0247]

The determined nucleotide sequence of the cDNA encoding the light chain variable region of A2-11E is shown in SEQ ID NO: 3 in the Sequence Listing, and the amino acid sequence is shown in SEQ ID NO: 4.

[0248]

3)-2 Nucleotide sequencing of cDNAs encoding variable regions of A2-15A

3)-2-1 Preparation of total RNA from A2-15A-producing hybridomas

To amplify cDNAs containing variable regions of A2-15A, total RNA was prepared from the A2-15A-producing hybridomas, as in Example 3)-1-1.

[0249]

3)-2-2 Synthesis of a cDNA (5'-RACE-Ready cDNA)

A cDNA (5'-RACE-Ready cDNA) was synthesized from 1 µg of the total RNA prepared in Example 3)-2-1, as in Example 3)-1-2.

[0250]

3)-2-3 Amplification by 5'-RACE PCR and sequencing of a cDNA containing a heavy chain variable region of A2-15A

A cDNA containing a heavy chain variable region of A2-15A was amplified as in Example 3)-1-3, using the cDNA (5'-RACE-Ready cDNA) synthesized in Example 3)-2-2 as a template, and the sequence was determined.

[0251]

The determined nucleotide sequence of the cDNA encoding the heavy chain variable region of A2-15A is shown in SEQ ID NO: 5 in the Sequence Listing, and the amino acid sequence is shown in SEQ ID NO: 6.

[0252]

3)-2-4 Amplification by 5'-RACE PCR and sequencing of a cDNA containing a light chain variable region of A2-15A

A cDNA containing a light chain variable region of A2-15A was amplified as in Example 3)-1-4, using the cDNA (5'-RACE-Ready cDNA) synthesized in Example 3)-2-2 as a template, and the sequence was determined.

[0253]

The determined nucleotide sequence of the cDNA encoding the light chain variable region of A2-15A is shown in SEQ ID NO: 7 in the Sequence Listing, and the amino acid sequence is shown in SEQ ID NO: 8.

[0254]

3)-3 Nucleotide sequencing of cDNAs encoding variable regions of A2-25C

3)-3-1 Preparation of total RNA from A2-25C-producing hybridomas

To amplify cDNAs containing variable regions of A2-25C, total RNA was prepared from the A2-25C-producing hybridomas, as in Example 3)-1-1.

[0255]

3)-3-2 Synthesis of a cDNA (5'-RACE-Ready cDNA)

A cDNA (5'-RACE-Ready cDNA) was synthesized from 1 µg of the total RNA prepared in Example 3)-3-1, as in Example 3)-1-2.

[0256]

3)-3-3 Amplification by 5'-RACE PCR and sequencing of a cDNA containing a heavy chain variable region of A2-25C

A cDNA containing a heavy chain variable region of A2-25C was amplified as in Example 3)-1-3, using the cDNA (5'-RACE-Ready cDNA) synthesized in Example 3)-3-2 as a template, and the sequence was determined.

[0257]

The determined nucleotide sequence of the cDNA encoding the heavy chain variable region of A2-25C is shown in SEQ ID NO: 9 in the Sequence Listing, and the amino acid sequence is shown in SEQ ID NO: 10.

[0258]

3)-3-4 Amplification by 5'-RACE PCR and sequencing of a cDNA containing a light chain variable region of A2-25C

A cDNA containing a light chain variable region of A2-25C was amplified as in Example 3)-1-4, using the cDNA (5'-RACE-Ready cDNA) synthesized in Example 3)-3-2 as a template, and the sequence was determined.

[0259]

The determined nucleotide sequence of the cDNA encoding the light chain variable region of A2-25C is shown in SEQ ID NO: 11 in the Sequence Listing, and the amino acid sequence is shown in SEQ ID NO: 12.

[0260]

3)-4 Nucleotide sequencing of cDNAs encoding variable regions of A2-27D

3)-4-1 Preparation of total RNA from A2-27D-producing hybridomas

To amplify cDNAs containing variable regions of A2-27D, total RNA was prepared from the A2-27D-producing hybridomas, as in Example 3)-1-1.

[0261]

3)-4-2 Synthesis of a cDNA (5'-RACE-Ready cDNA)

A cDNA (5'-RACE-Ready cDNA) was synthesized from 1 µg of the total RNA prepared in Example 3)-4-1, as in Example 3)-1-2.

[0262]

3)-4-3 Amplification by 5'-RACE PCR and sequencing of a cDNA containing a heavy chain variable region of A2-27D

A cDNA containing a heavy chain variable region of A2-27D was amplified as in Example 3)-1-3, using the cDNA (5'-RACE-Ready cDNA) synthesized in Example 3)-4-2 as a template, and the sequence was determined.

[0263]

The determined nucleotide sequence of the cDNA encoding the heavy chain variable region of A2-27D is shown in SEQ ID NO: 13 in the Sequence Listing, and the amino acid sequence is shown in SEQ ID NO: 14.

[0264]

3)-4-4 Amplification by 5'-RACE PCR and sequencing of a cDNA containing a light chain variable region of A2-27D

A cDNA containing a light chain variable region of A2-27D was amplified as in Example 3)-1-4, using the cDNA (5'-RACE-Ready cDNA) synthesized in Example 3)-4-2 as a template, and the sequence was determined.

[0265]

The determined nucleotide sequence of the cDNA encoding the light chain variable region of A2-27D is shown in SEQ ID NO: 15 in the Sequence Listing, and the amino acid sequence is shown in SEQ ID NO: 16.

[0266]

<Example 4>

In vivo evaluation of rat anti-ALK2 antibodies (A2-15A and A2-27D)

4)-1 Preparation of hybridoma culture supernatants

1.0×10^6 hybridomas obtained in Example 1)-4 were cultured in 10% FBS-containing TIL high-glucose medium (T75 flask), and then cultured at high density using INTEGRA CL1000 (10% FBS medium). Next, the medium was replaced with a serum-free medium, the cells were further cultured in INTEGRA CL1000, and then required volumes of hybridoma culture supernatants were obtained. The obtained hybridoma culture supernatants were stored at 2 to 8 °C until they were subjected to purification.

[0267]

4)-2 Purification of the antibodies from the hybridoma culture supernatants

The antibodies were purified from the culture supernatants obtained in Example 4)-1 by protein G affinity chromatography (at 4 to 6 °C) in one step. The buffer replacement step after the purification by protein G affinity chromatography was performed at 4 to 6 °C. Initially, each culture supernatant of hybridomas was applied to a PBS-equilibrated column packed with protein G (GE Healthcare Bioscience). After the entry of the whole culture supernatant in the column, the column was washed with at least twice the column volume of PBS. Next, fractions containing the antibody were collected by elution with a 0.1 M aqueous glycine/hydrochloric acid solution (pH 2.7). The collected fractions were adjusted to pH 7.0 to 7.5 by the addition of 1 M Tris-HCl (pH 9.0), and then subjected to buffer replacement with HBSor (25 mM histidine/5% sorbitol, pH 6.0) by dialysis (Thermo Scientific, Slide-A-Lyzer Dialysis Cassette). The fractions were concentrated using Centrifugal UF Filter Device VIVASPIN20 (molecular weight cutoff: UF10K, Sartorius, at 4 °C), and adjusted to an IgG concentration of 8 mg/ml or more. Finally, the fractions were filtered using the Minisart-Plus filter (Sartorius) to obtain a purified sample.

[0268]

4)-3 BMP transplantation-induced heterotopic ossification induction models

The heterotopic ossification-suppressing activity of the monoclonal antibodies in skeletal muscle tissue was analyzed by BMP-induced heterotopic ossification induction experiments in mice. BMP-containing pellets were prepared by impregnating a type I collagen sponge (Collatape, Zimmer Dental) adjusted to a diameter of 4 mm with 1 µg of BMP7 (Miltene) or 1 µg of GDF2/BMP9 (Peprotech), and freeze-drying the sponge overnight in a freeze-drying machine (FDU-810, Tokyo Rikakikai Co., Ltd., Japan). 8- to 10-week-old C57BL/6 mice were placed under general anesthesia, using a simple inhalation anesthesia apparatus for small animal experiments (Natsume Seisakusho Co., Ltd., Japan) and 2% isoflurane (Wako Pure Chemical Industries, Ltd., Japan), and one of the BMP-containing pellets was transplanted into the thigh muscle tissue of each of the left and right lower extremities. The mice received subcutaneous administration of the monoclonal antibodies produced by hybridomas A2-15A and A2-27D at 10 mg/kg once a week, from 1 week before the transplantation until week 2 after the transplantation, as they were raised. An equal volume of a solvent (25 mM Histidine/5% Sorbitol, pH 6.0) was administered to a control group. Two weeks after the transplantation of the BMP-containing pellets, the thigh bone with the transplanted BMP-containing pellets were extracted, and heterotopic ossification was analyzed by micro-CT (µCT35, SCANCO).

[0269]

The results are shown in Figure 8. The micro-CT analysis showed that heterotopic ossification was induced on the right-hand side of the thigh bone in the mice transplanted with BMP7 and GDF2/BMP9 and treated with the vehicle. In contrast, heterotopic ossification was not detected in the mice that received the administration of the monoclonal antibodies produced by hybridomas A2-15A and A2-27D, although they were transplanted with BMP7 and GDF2/BMP9. It was thus confirmed that the monoclonal antibodies produced by hybridomas A2-15A and A2-27D suppressed the induction of heterotopic ossification in the skeletal muscle tissue induced by BMP7 and GDF2/BMP9.

[0270]

<Example 5>

Preparation of human chimeric anti-ALK2 antibodies (cA2-15A and cA2-27D)

5)-1 Construction of a chimeric and humanized antibody light chain expression vector pCMA-LK

A fragment of about 5.4 kb obtained by digesting the plasmid pcDNA3.3-TOPO/LacZ (Invitrogen) with restriction enzymes XbaI and PmeI and a DNA fragment containing a DNA sequence encoding a human κ chain secretion signal and a human κ chain constant region shown in the SEQ ID NO: 17 were ligated using In-Fusion Advantage PCR Cloning Kit (Clontech) to prepare pcDNA3.3/LK.

[0271]

pcDNA3.3/LK was used as a template in PCR using the following primer set. The obtained fragment of about 3.8 kb was phosphorylated and then self-ligated, thereby constructing a chimeric and humanized antibody light chain expression vector pCMA-LK having a signal sequence, a cloning site, and a human κ chain constant region downstream of the CMV promoter.

Primer set:

5'-TATACCGTCGACCTCTAGCTAGAGCTTGGC-3' (3.3-F1; SEQ ID NO: 90)

5'-GCTATGGCAGGGCCTGCCGCCCCGACGTTG-3' (3.3-R1; SEQ ID NO: 91)

[0272]

5)-2 Construction of a chimeric and humanized antibody IgG1 type heavy chain expression vector pCMA-G1

A DNA fragment lacking the human κ chain secretion signal and the human κ chain constant region by digesting pCMA-LK with XbaI and PmeI and a DNA fragment containing a DNA sequence encoding amino acids of a human heavy chain signal sequence and a human IgG1 constant region of SEQ ID NO: 18 were ligated using In-Fusion Advantage PCR Cloning Kit (Clontech), thereby constructing a chimeric and humanized antibody IgG1 type heavy chain expression vector pCMA-G1 having a signal sequence, a cloning site, and a human IgG1 heavy chain constant region downstream of the CMV promoter.

[0273]

5)-3 Construction of a cA2-15A heavy chain expression vector

Using the cDNA containing the heavy chain variable region of A2-15A obtained in Example 3)-2-3 as a template, a DNA fragment containing the cDNA encoding the heavy chain variable region was amplified, using KOD -Plus- (TOYOBO, Japan) and the following primer set, and inserted into a restriction enzyme BlnI-cleaved site of the chimeric and humanized IgG1 type heavy chain expression vector pCMA-G1, using In-Fusion HD PCR Cloning Kit (Clontech), thereby constructing a cA2-15A heavy chain expression vector. The obtained expression vector was designated as "pCMA-G1/cA2-15A". The nucleotide sequence of the cA2-15A heavy chain is shown in SEQ ID NO: 19, and the amino acid sequence is shown in SEQ ID NO: 20.

Primer set for the cA2-15A heavy chain:

5'-CCAGATGGGTGCTGAGCGAGGTGCAGCTGGTGGAGTCTGGCGGAG-3'

(A2-15AH-F; SEQ ID NO: 92)

5'-CTTGGTGGAGGCTGAGCTGACAGTGACCAGAGTGCCTTGGCCCCAG-3'

(A2-15AH-R; SEQ ID NO: 93)

[0274]

5)-4 Construction of a cA2-15A light chain expression vector

Using the cDNA containing the light chain variable region of A2-15A obtained in Example 3)-2-4 as a template, a DNA fragment containing the cDNA encoding the light chain variable region was amplified, using KOD -Plus- (TOYOBO, Japan) and the following primer set, and inserted into a restriction enzyme BsiWI-cleaved site of the chimeric and humanized light chain expression general-purpose vector pCMA-LK, using In-Fusion HD PCR Cloning Kit (Clontech), thereby constructing a cA2-15A light chain expression vector. The obtained expression vector was designated as "pCMA-LK/cA2-15A". The nucleotide sequence of the cA2-15A light chain is shown in SEQ ID NO: 21 in the Sequence Listing, and the amino acid sequence is shown in SEQ ID NO: 22.

Primer set for the cA2-15A light chain:

5'-ATCTCCGGCGCGTACGGCGACATTGTCTTGACCCAGTCTCCTGC-3' (A2-

15AL-F; SEQ ID NO: 94)

5'-GGAGGGGGCGGCCACAGCCCGTTTCAGTTCCAGCTTGGTCCCAG-3' (A2-15AL-R; SEQ ID NO: 95)

[0275]

5)-5 Construction of a cA2-27D heavy chain expression vector

Using the cDNA containing the heavy chain variable region of A2-27D obtained in Example 3)-4-3 as a template, a DNA fragment containing the cDNA encoding the heavy chain variable region was amplified using KOD -Plus- (TOYOBO, Japan) and the following primer set, and inserted into a restriction enzyme BspI-cleaved site of the chimeric and humanized IgG1 type heavy chain expression vector pCMA-G1, using In-Fusion HD PCR Cloning Kit (Clontech), thereby constructing a cA2-27D heavy chain expression vector. The obtained expression vector was designated as "pCMA-G1/cA2-27D". The nucleotide sequence of the cA2-27D heavy chain is shown in SEQ ID NO: 23 in the Sequence Listing, and the amino acid sequence is shown in SEQ ID NO: 24.

Primer set for the cA2-27D heavy chain:

5'-CCAGATGGGTGCTGAGCGAGGTGCAGCTGGTGGAGTCTGGAGGAG-3'
(A2-27DH-F; SEQ ID NO: 96)

5'-CTTGGTGGAGGCTGAGCTCACGGTGACCACGGTTCCTGGGCCCCAG-3'
(A2-27DH-R; SEQ ID NO: 97)

[0276]

5)-6 Construction of a cA2-27D light chain expression vector

Using the cDNA containing the light chain variable region of A2-27D obtained in Example 3)-4-4 as a template, a DNA fragment containing the cDNA encoding the light chain variable region was amplified using KOD -Plus- (TOYOBO, Japan) and the following primer set, and inserted into a restriction enzyme BspWI-cleaved site of the chimeric and humanized antibody light chain expression general-purpose vector pCMA-LK, using In-Fusion HD PCR Cloning Kit (Clontech), thereby constructing a cA2-27D antibody light chain expression vector. The obtained expression vector was designated as "pCMA-LK/cA2-27D". The nucleotide sequence of the cA2-27D antibody light chain is shown in SEQ ID NO: 25, and the amino acid sequence is shown in SEQ ID NO: 26.

Primer set for the cA2-27D light chain:

5'-ATCTCCGGCGCGTACGGCGAAATTGTTCTCACTCAGTCTCCAAC-3' (A2-27DL-F; SEQ ID NO: 98)

5'-GGAGGGGGCGGCCACAGCCCGTTTCAGTTCCAGCTTGGTCCCAG-3' (A2-15AL-R; SEQ ID NO: 95)

[0277]

5)-7 Production of cA2-15A and cA2-27D

FreeStyle 293F cells (Invitrogen) were subcultured and cultured in accordance with the manual. 1.2×10^9 FreeStyle 293F cells (Invitrogen) in the logarithmic growth phase were seeded into 3 L Fernbach Erlenmeyer Flask (Corning), and diluted with the FreeStyle293 expression medium (Invitrogen) to 2.0×10^6 cells/ml, and then the cells were cultured with shaking at 90 rpm for 1 hour in an 8% CO₂ incubator at 37 °C. 1.8 mg of polyethyleneimine (Polyscience #24765) was dissolved in 20 ml of the Opti-Pro SFM medium (Invitrogen). Then, the H chain expression vector (0.24 mg) and the L chain expression vector (0.36 mg) prepared using NucleoBond Xtra (TaKaRa, Japan) were added into 20 ml of the Opti-Pro SFM medium (Invitrogen). 20 ml of the expression vector/Opti-Pro SFM mixed solution was added into 20 ml of the polyethyleneimine/Opti-Pro SFM mixed solution, and the mixture was gently stirred, further left for 5 minutes, and then added into the FreeStyle 293F cells. A culture supernatant obtained by shake culture at 90 rpm for 4 hours in an 8% CO₂ incubator at 37 °C, followed by the addition of 600 ml of the EX-CELL VPRO medium (SAFC Biosciences), 18 ml of GlutaMAX I (GIBCO), and 30 ml of Yeastolate Ultrafiltrate (GIBCO), and by shake culture at 90 rpm for 7 days in an 8% CO₂ incubator at 37°C, was filtered through Disposable Capsule Filter (Advantec #CCS-045-E1H).

[0278]

The chimeric antibody of the rat antibody A2-15A obtained by the combination of pCMA-G1/cA2-15A and pCMA-LK/cA2-15A was designated as "cA2-15A", and the chimeric antibody of the rat antibody A2-27D obtained by the combination of pCMA-G1/cA2-27D and pCMA-LK/cA2-27D was designated as "cA2-27D".

[0279]

5)-8 Two-step purification of cA2-15A and cA2-27D

Each of the antibodies was purified from the culture supernatant obtained in Example 5)-7 by two steps using rProtein A affinity chromatography (at 4 to 6 °C) and ceramic hydroxyapatite (at room temperature). Buffer replacement steps after the rProtein A affinity chromatography purification and after the ceramic hydroxyapatite purification were performed at 4 to 6 °C. The culture supernatant was applied to MabSelectSuRe (GE Healthcare Bioscience, HiTrap column) equilibrated with PBS. After the entry of the whole culture supernatant in the column, the column was washed with at least twice the column volume of PBS. Next, fractions containing the antibody were collected by elution with a 2 M arginine hydrochloride solution (pH 4.0). The fractions were subjected to buffer replacement with PBS by dialysis (Thermo Scientific, Slide-A-Lyzer Dialysis Cassette), and then diluted 5-fold with 5 mM sodium phosphate/50 mM MES/pH 7.0 buffer, and the resulting antibody solution was applied to a ceramic hydroxyapatite column (Bio-Rad Japan, Bio-Scale CHT Type-1 Hydroxyapatite Column) equilibrated with 5 mM NaPi/50 mM MES/30 mM NaCl/pH 7.0 buffer. The fractions containing the antibody were collected by performing linear concentration gradient elution with sodium chloride. The fractions were subjected to buffer replacement with HBSor (25 mM histidine/5% sorbitol, pH 6.0) by dialysis (Thermo Scientific, Slide-A-Lyzer Dialysis Cassette). The fractions were concentrated using Centrifugal UF Filter Device VIVASPIN20 (molecular weight cutoff: UF10K, Sartorius, at 4 °C), and adjusted to an IgG concentration of 2 mg/ml or more. Finally, the fractions were filtered using the Minisart-Plus filter (Sartorius) to obtain a purified sample.

[0280]

<Example 6>

Evaluation of the *in vitro* activity of human chimeric anti-ALK2 antibodies (cA2-15A and cA2-27D)

6)-1 Evaluation of the antibodies by luciferase reporter assay

The ALK2-mediated intracellular signaling inhibitory activity of each of the human chimeric antibodies prepared was analyzed using a BMP-specific luciferase reporter. HEPG2 cells were seeded into a 96-well white plate for luciferase assay (Corning) at 1×10^4

cells/well, and cultured overnight in 10% FBS-containing DMEM medium under the conditions of 5% CO₂ at 37 °C. On the following day, the HEPG2 cells were transfected with pGL4.26/Id1WT4F-luc (Genes Cells, 7, 949 (2002)), using Lipofectamine 2000 (Invitrogen). After 2.5 hours, the medium was replaced with fresh OPTI-MEM I (LifeTechnologies), and the cells were further cultured for 3 hours. Thereafter, the medium was replaced with OPTI-MEM I containing the serially diluted monoclonal antibody and 10 ng/mL of BMP7 (Milteneu), and the cells were further cultured overnight. On the following day, the luciferase activity was measured with the plate reader SpectraMaxM4 (Molecular Devices), using Dual-Glo Luciferase Assay System (Promega).

[0281]

The results are shown in Figure 9. Each of the chimeric antibodies cA2-15A and cA2-27D was confirmed to demonstrate an inhibitory activity comparable to that of the rat monoclonal antibody A2-15A or A2-27D, respectively, upon the BMP7-induced BMP-specific luciferase activity.

[0282]

6)-2 Evaluation of the antibodies by BMP-induced osteoblast differentiation assay

The inhibitory activity of each of the human chimeric antibodies against endogenous ALK2-mediated intracellular signaling was analyzed based on the effect upon the BMP-induced osteoblast differentiation of C2C12 cells. C2C12 cells were seeded into a 96-well plate (Iwaki & Co., Ltd.) at 5×10^3 cells/well, and cultured in 15% FBS-containing DMEM medium overnight under the conditions of 5% CO₂ at 37 °C. On the following day, the medium was replaced with fresh OPTI-MEM I (LifeTechnologies) containing the serially diluted monoclonal antibody and 2 ng/mL of GDF2/BMP9 (Peprotech), and the cells were further cultured for 3 days. After the medium was removed from the C2C12 cells and the cells were washed with PBS, the cells were treated with 50 µL/well of an ice-cold acetone:ethanol (1:1) solution for 1 minute, and further washed with PBS three times. ALP activity was measured as an index of the differentiation into cells for forming osteoblasts. To measure ALP activity, 100 µL/well of a substrate solution (0.1 M diethanolamine (Sigma-Aldrich)-HCl, pH 10.0, containing 1 mg/mL of 4-nitrophenyl phosphate (Sigma-Aldrich) and

1 mM of MgCl_2) was added, and reacted for 15 to 30 minutes at room temperature on an agitation shaker. The reaction was stopped by the addition of 50 μL of 3M NaOH, and absorbance at a wavelength of 405 nm was measured using the microplate reader SpectraMax M4 (Molecular Devices).

[0283]

The results are shown in Figure 10. It was confirmed that the chimeric antibodies cA2-15A and cA2-27D suppressed the BMP-induced differentiation of the C2C12 cells into osteoblast-like cells in a dose-dependent manner. These results show that the chimeric antibodies cA2-15A and cA2-27D are antibodies that suppress endogenous ALK2 physiologically expressed by the C2C12 cells. Moreover, the inhibitory activity of each of the chimeric antibodies cA2-15A and cA2-27D was confirmed to be comparable to that of the rat monoclonal antibody A2-15A or A2-27D, respectively.

[0284]

<Example 7>

Design of humanized versions of anti-ALK2 antibodies 2-15A and A2-27D

7)-1 Design of humanized hA2-15

7)-1-1 Molecular modeling of the variable regions of A2-15A

The molecular modeling of the variable regions of A2-15A was performed using a method known as homology modeling (Methods in Enzymology, 203, 121-153, (1991)). The variable regions of A2-15A determined above were compared with the primary sequences of human immunoglobulin variable regions registered in Protein Data Bank (three-dimensional structures derived from X-ray crystal structures are available) (Nuc. Acid Res. 35, D301-D303 (2007)). As a result, 3KYM and 3S35 were selected as those having the highest sequence identity to the heavy chain variable region and the light chain variable region, respectively, of A2-15A. The three-dimensional structures of framework regions were prepared by obtaining a "framework model" by combining the coordinates of 3KYM and 3S35 corresponding to the heavy chain and the light chain of A2-15A. Then, a representative conformation of each CDR was incorporated into the framework model.

[0285]

Finally, energy calculation for excluding disadvantageous interatomic contact was performed, in order to obtain possible molecular models of the variable regions of A2-15A in terms of energy. The above-described procedures were performed using a commercially available protein three-dimensional structure analysis program, Discovery Studio (Accelrys, Inc.).

[0286]

7)-1-2 Design of an amino acid sequence for humanized hA2-15A

The humanized hA2-15 was constructed using a method commonly known as CDR grafting (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). An acceptor antibody was selected based on the amino acid identity in framework regions.

[0287]

The sequences of the framework regions of A2-15 were compared with the sequences of framework regions of human subgroup consensus sequences. As a result, the human γ chain subgroup 3 consensus sequence and the human κ chain subgroup 4 consensus sequence defined by KABAT et al. (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service National Institutes of Health, Bethesda, MD. (1991)) were selected as acceptors due to their high sequence identity in framework regions. The amino acid residues of the framework regions in the human γ chain subgroup 3 consensus sequence and the human κ chain subgroup 4 consensus sequence were aligned with the amino acid residues of the framework regions of A2-15A to identify the numbers of amino acids differing therebetween. The numbers of these residues were analyzed using the three-dimensional model of A2-15A constructed above. Then, the donor residues to be grafted onto the acceptors were selected in accordance with the criteria provided by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). Some donor residues thus selected were transferred into the acceptor antibody to construct humanized hA2-15A sequences, as described in the following Examples.

[0288]

7)-2 Humanization of A2-15A heavy chains

7)-2-1 Humanized hA2-15A-H1 type heavy chain

A humanized hA2-15A heavy chain designed by substitution of amino acid number 35 (arginine) with glycine, amino acid number 38 (lysine) with arginine, amino acid number 61 (threonine) with glycine, amino acid number 94 (alanine) with serine, amino acid number 96 (serine) with asparagine, amino acid number 103 (aspartic acid) with asparagine, amino acid number 107 (serine) with alanine, amino acid number 112 (threonine) with valine, and amino acid number 116 (threonine) with alanine in the heavy chain of chimeric cA2-15A shown in SEQ ID NO: 20 was designated as "humanized hA2-15A-H1 type heavy chain" (sometimes also referred to as "hA2-15A-H1").

[0289]

The amino acid sequence of the humanized hA2-15A-H1 type heavy chain is described in SEQ ID NO: 28 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 19, a sequence consisting of amino acid numbers 20 to 142, and a sequence consisting of amino acid numbers 143 to 472 in the amino acid sequence of SEQ ID NO: 28 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 28 is described in SEQ ID NO: 27 in the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 57, a sequence consisting of nucleotide numbers 58 to 426, and a sequence consisting of nucleotide numbers 427 to 1416 in the nucleotide sequence of SEQ ID NO: 27 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 27 and the amino acid sequence of SEQ ID NO: 28 are also described in Figure 15.

[0290]

7)-2-2 Humanized hA2-15A-H4 type heavy chain

A humanized hA2-15A heavy chain designed by substitution of amino acid number 35 (arginine) with glycine, amino acid number 38 (lysine) with arginine, amino acid number 61 (threonine) with glycine, amino acid number 103 (aspartic acid) with asparagine, and amino acid number 107 (serine) with alanine in the heavy chain of chimeric cA2-15A shown in SEQ ID NO: 20 was designated as "humanized hA2-15A-H4 type heavy chain" (sometimes also referred to as "hA2-15A-H4").

[0291]

The amino acid sequence of the humanized hA2-15A-H4 type heavy chain is described in SEQ ID NO: 30 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 19, a sequence consisting of amino acid numbers 20 to 142, and a sequence consisting of amino acid numbers 143 to 472 in the amino acid sequence of SEQ ID NO: 30 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 30 is described in SEQ ID NO: 29 in the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 57, a sequence consisting of nucleotide numbers 58 to 426, and a sequence consisting of nucleotide numbers 427 to 1416 in the nucleotide sequence of SEQ ID NO: 29 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 29 and the amino acid sequence of SEQ ID NO: 30 are also described in Figure 16.

[0292]

7)-3 Humanization of A2-15A light chain

7)-3-1 Humanized hA2-15A-L1 type light chain

A humanized hA2-15A light chain designed by the substitution of amino acid number 24 (leucine) with methionine, amino acid number 29 (alanine) with aspartic acid, a position (missing residue) between amino acid numbers 29 and 30 with serine, amino acid number 36 (glutamine) with glutamic acid, amino acid number 41 (serine) with asparagine, amino acid number 66 (lysine) with proline, amino acid number 81 (isoleucine) with valine, amino acid number 83 (alanine) with aspartic acid, amino acid number 99 (asparagine) with serine, amino acid number 100 (proline) with serine, amino acid number 101 (valine) with leucine, amino acid number 104 (aspartic acid) with glutamic acid, amino acid number 106 (isoleucine) with valine, amino acid number 108 (threonine) with valine, amino acid number 123 (alanine) with glutamine, amino acid number 127 (leucine) with valine, and amino acid number 129 (leucine) with isoleucine in the light chain of chimeric cA2-15A shown in SEQ ID NO: 22 was designated as "humanized hA2-15A-L1 type light chain" (sometimes also referred to as "hA2-15A-L1").

[0293]

The amino acid sequence of the humanized hA2-15A-L1 type light chain is described in SEQ ID NO: 32 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20, a sequence consisting of amino acid numbers 21 to 133, and a sequence consisting of amino acid numbers 134 to 238 in the amino acid sequence of SEQ ID NO: 32 correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 32 is described in SEQ ID NO: 31 in the Sequence Listing. A sequence consisting of nucleotide numbers 26 to 85, a sequence consisting of nucleotide numbers 86 to 424, and a sequence consisting of nucleotide numbers 425 to 739 in the nucleotide sequence of SEQ ID NO: 31 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 31 and the amino acid sequence of SEQ ID NO: 32 are also described in Figure 17.

[0294]

7)-3-2 Humanized hA2-15A-L4 type light chain

A humanized hA2-15A light chain designed by the substitution of amino acid number 29 (alanine) with aspartic acid, a position (missing residue) between amino acid numbers 29 and 30 with serine, amino acid number 36 (glutamine) with glutamic acid, amino acid number 41 (serine) with asparagine, amino acid number 99 (asparagine) with serine, amino acid number 100 (proline) with serine, amino acid number 108 (threonine) with valine, and amino acid number 123 (alanine) with glutamine in the light chain of chimeric cA2-15A shown in SEQ ID NO: 22 was designated as "humanized hA2-15A-L4 type light chain" (sometimes also referred to as "hA2-15A-L4").

[0295]

The amino acid sequence of the humanized hA2-15A-L4 type light chain is described in SEQ ID NO: 34 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20, a sequence consisting of amino acid numbers 21 to 133, and a sequence consisting of amino acid numbers 134 to 238 in the amino acid sequence of SEQ ID NO: 34 correspond to the signal sequence, the light chain variable region, and the light chain constant region,

respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 34 is described in SEQ ID NO: 33 in the Sequence Listing. A sequence consisting of nucleotide numbers 26 to 85, a sequence consisting of nucleotide numbers 86 to 424, and a sequence consisting of nucleotide numbers 425 to 739 in the nucleotide sequence of SEQ ID NO: 33 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 33 and the amino acid sequence of SEQ ID NO: 34 are also described in Figure 18.

[0296]

7)-3-3 Humanized hA2-15A-L6 type light chain

A humanized hA2-15A light chain designed by the substitution of amino acid number 29 (alanine) with aspartic acid, a position (missing residue) between amino acid numbers 29 and 30 with serine, amino acid number 36 (glutamine) with glutamic acid, amino acid number 41 (serine) with asparagine, amino acid number 79 (serine) with glutamine, amino acid number 99 (asparagine) with serine, amino acid number 100 (proline) with serine, amino acid number 108 (threonine) with valine, and amino acid number 123 (alanine) with glutamine in the light chain of chimeric cA2-15A shown in SEQ ID NO: 22 was designated as "humanized hA2-15A-L6 type light chain" (sometimes also referred to as "hA2-15A-L6").

[0297]

The amino acid sequence of the humanized hA2-15A-L6 type light chain is described in SEQ ID NO: 36 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20, a sequence consisting of amino acid numbers 21 to 133, and a sequence consisting of amino acid numbers 134 to 238 in the amino acid sequence of SEQ ID NO: 36 correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 36 is described in SEQ ID NO: 35 in the Sequence Listing. A sequence consisting of nucleotide numbers 26 to 85, a sequence consisting of nucleotide numbers 86 to 424, and a sequence consisting of nucleotide numbers 425 to 739 in the nucleotide sequence of SEQ ID NO: 35 encode the signal sequence, the light chain variable region sequence, and the light chain

constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 35 and the amino acid sequence of SEQ ID NO: 36 are also described in Figure 19.

[0298]

7)-3-4 Humanized hA2-15A-L7 type light chain

A humanized hA2-15A light chain designed by the substitution of amino acid number 29 (alanine) with aspartic acid, a position (missing residue) between amino acid numbers 29 and 30 with serine, amino acid number 36 (glutamine) with glutamic acid, amino acid number 41 (serine) with asparagine, amino acid number 70 (leucine) with alanine, amino acid number 99 (asparagine) with serine, amino acid number 100 (proline) with serine, amino acid number 108 (threonine) with valine, and amino acid number 123 (alanine) with glutamine in the light chain of chimeric cA2-15A shown in SEQ ID NO: 22 was designated as "humanized hA2-15A-L7 type light chain" (sometimes also referred to as "hA2-15A-L7").

[0299]

The amino acid sequence of the humanized hA2-15A-L7 type light chain is described in SEQ ID NO: 38 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20, a sequence consisting of amino acid numbers 21 to 133, and a sequence consisting of amino acid numbers 134 to 238 in the amino acid sequence of SEQ ID NO: 38 correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 38 is described in SEQ ID NO: 37 in the Sequence Listing. A sequence consisting of nucleotide numbers 26 to 85, a sequence consisting of nucleotide numbers 86 to 424, and a sequence consisting of nucleotide numbers 425 to 739 in the nucleotide sequence of SEQ ID NO: 37 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 37 and the amino acid sequence of SEQ ID NO: 38 are also described in Figure 20.

[0300]

7)-4 Design of humanized hA2-15A by combinations of heavy chains and light chains

An antibody consisting of the humanized hA2-15A-H1 type heavy chain and the humanized hA2-15A-L1 type light chain was designed and designated as "humanized hA2-

15A-H1/L1" (sometimes also referred to as "hA2-15A-H1/L1"). An antibody consisting of the humanized hA2-15A-H1 type heavy chain and the humanized hA2-15A-L4 type light chain was designed and designated as "humanized hA2-15A-H1/L4" (sometimes also referred to as "hA2-15A-H1/L4"). An antibody consisting of the humanized hA2-15A-H4 type heavy chain and the humanized hA2-15A-L1 type light chain was designed and designated as "humanized hA2-15A-H4/L1" (sometimes also referred to as "hA2-15A-H4/L1"). An antibody consisting of the humanized hA2-15A-H4 type heavy chain and the humanized hA2-15A-L4 type light chain was designed and designated as "humanized hA2-15A-H4/L4" (sometimes also referred to as "hA2-15A-H4/L4"). An antibody consisting of the humanized hA2-15A-H4 type heavy chain and the humanized hA2-15A-L6 type light chain was designed and designated as "humanized hA2-15A-H4/L6" (sometimes also referred to as "hA2-15A-H4/L6"). An antibody consisting of the humanized hA2-15A-H4 type heavy chain and the humanized hA2-15A-L7 type light chain was designed and designated as "humanized hA2-15A-H4/L7" (sometimes also referred to as "hA2-15A-H4/L7"). The antibodies designed above can be prepared in accordance with Example 8, and evaluated in accordance with Examples 2 and 4.

[0301]

7)-5 Design of humanized hA2-27D

7)-5-1 Molecular modeling of the variable regions of A2-27D

The molecular modeling of the variable regions of A2-27D was performed using a method known as homology modeling (Methods in Enzymology, 203, 121-153, (1991)). The variable regions of A2-27D determined above were compared with the primary sequences of human immunoglobulin variable regions registered in Protein Data Bank (three-dimensional structures derived from X-ray crystal structures are available) (Nuc. Acid Res. 35, D301-D303 (2007)). As a result, 3EYQ and 4I9W were selected as those having the highest sequence identity to the heavy chain variable region and the light chain variable region, respectively, of A2-27D. The three-dimensional structures of framework regions were prepared by obtaining a "framework model" by combining the coordinates of 3EYQ and 4I9W corresponding to the

heavy chain and the light chain of A2-27D. Then, a representative conformation of each CDR was incorporated into the framework model.

[0302]

Finally, energy calculation for excluding disadvantageous interatomic contact was performed, in order to obtain possible molecular models of the variable regions of A2-27D in terms of energy. The above-described procedures were performed using a commercially available protein three-dimensional structure analysis program, Discovery Studio (Accelrys, Inc.).

[0303]

7)-5-2 Design of an amino acid sequence for humanized hA2-27D

The humanized hA2-27D was constructed using a method commonly known as CDR grafting (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). An acceptor antibody was selected based on the amino acid identity in framework regions.

[0304]

The sequences of the framework regions of A2-27D were compared with the sequences of framework regions of human subgroup consensus sequences. As a result, the human γ chain subgroup 3 consensus sequence and the human κ chain subgroup 4 consensus sequence defined by KABAT et al. (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service National Institutes of Health, Bethesda, MD. (1991)) were selected as acceptors due to their high sequence identity in framework regions. The amino acid residues of the framework regions in the human γ chain subgroup 3 consensus sequence and the human κ chain subgroup 3 consensus sequence were aligned with the amino acid residues of the framework regions of A2-27D to identify the numbers of amino acids differing therebetween. The numbers of these residues were analyzed using the three-dimensional model of A2-27D constructed above. Then, the donor residues to be grafted onto the acceptors were selected in accordance with the criteria provided by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). Some donor residues thus selected were transferred into the acceptor antibody to construct humanized hA2-27D sequences, as described in the following Examples.

[0305]

7)-6 Humanization of A2-27D heavy chains

7)-6-1 Humanized hA2-27D-H1 type heavy chain

A humanized hA2-15A heavy chain designed by the substitution of amino acid number 35 (arginine) with glycine, amino acid number 38 (lysine) with arginine, amino acid number 42 (leucine) with alanine, amino acid number 56 (isoleucine) with valine, amino acid number 68 (alanine) with serine, amino acid number 94 (alanine) with serine, amino acid number 95 (arginine) with lysine, amino acid number 103 (threonine) with asparagine, amino acid number 107 (serine) with alanine, amino acid number 112 (leucine) with valine, amino acid number 117 (alanine) with arginine, amino acid number 132 (proline) with glutamine, and amino acid number 135 (valine) with leucine in the heavy chain of chimeric cA2-27D shown in SEQ ID NO: 24 was designated as "humanized hA2-27D-H1 type heavy chain" (sometimes also referred to as "hA2-27D-H1").

[0306]

The amino acid sequence of the humanized hA2-27D-H1 type heavy chain is described in SEQ ID NO: 40 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 19, a sequence consisting of amino acid numbers 20 to 140, and a sequence consisting of amino acid numbers 141 to 470 in the amino acid sequence of SEQ ID NO: 40 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 40 is described in SEQ ID NO: 39 in the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 57, a sequence consisting of nucleotide numbers 58 to 420, and a sequence consisting of nucleotide numbers 421 to 1410 in the nucleotide sequence of SEQ ID NO: 39 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 39 and the amino acid sequence of SEQ ID NO: 40 are also described in Figure 21.

[0307]

7)-6-2 Humanized hA2-27D-H2 type heavy chain

A humanized hA2-27D heavy chain designed by the substitution of amino acid number 35 (arginine) with glycine, amino acid number 38 (lysine) with arginine, amino acid number

42 (leucine) with alanine, amino acid number 68 (alanine) with serine, amino acid number 94 (alanine) with serine, amino acid number 95 (arginine) with lysine, amino acid number 103 (threonine) with asparagine, amino acid number 107 (serine) with alanine, amino acid number 112 (leucine) with valine, amino acid number 132 (proline) with glutamine, and amino acid number 135 (valine) with leucine in the heavy chain of chimeric cA2-27D shown in SEQ ID NO: 24 was designated as "humanized hA2-27D-H2 type heavy chain" (sometimes also referred to as "hA2-27D-H2").

[0308]

The amino acid sequence of the humanized hA2-27D-H2 type heavy chain is described in SEQ ID NO: 42 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 19, a sequence consisting of amino acid numbers 20 to 140, and a sequence consisting of amino acid residues 141 to 470 in the amino acid sequence of SEQ ID NO: 42 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 42 is described in SEQ ID NO: 41 in the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 57, a sequence consisting of nucleotide numbers 58 to 420, and a sequence consisting of nucleotide numbers 421 to 1410 in the nucleotide sequence of SEQ ID NO: 41 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 41 and the amino acid sequence of SEQ ID NO: 42 are also described in Figure 22.

[0309]

7)-6-3 Humanized hA2-27D-H3 type heavy chain

A humanized hA2-27D heavy chain designed by the substitution of amino acid number 35 (arginine) with glycine, amino acid number 38 (lysine) with arginine, amino acid number 42 (leucine) with alanine, amino acid number 94 (alanine) with serine, amino acid number 95 (arginine) with lysine, amino acid number 103 (threonine) with asparagine, amino acid number 107 (serine) with alanine, amino acid number 112 (leucine) with valine, amino acid number 132 (proline) with glutamine, and amino acid number 135 (valine) with leucine in the heavy

chain of chimeric cA2-27D shown in SEQ ID NO: 24 was designated as "humanized hA2-27D-H3 type heavy chain" (sometimes also referred to as "hA2-27D-H3").

[0310]

The amino acid sequence of the humanized hA2-27D-H3 type heavy chain is described in SEQ ID NO: 44 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 19, a sequence consisting of amino acid numbers 20 to 140, and a sequence consisting of amino acid numbers 141 to 470 in the amino acid sequence of SEQ ID NO: 44 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 44 is described in SEQ ID NO: 43 in the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 57, a sequence consisting of nucleotides 58 to 420, and a sequence consisting of nucleotide numbers 421 to 1410 in the nucleotide sequence of SEQ ID NO: 43 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 43 and the amino acid sequence of SEQ ID NO: 44 are also described in Figure 23.

[0311]

7)-6-4 Humanized hA2-27D-H4 type heavy chain

A humanized hA2-27D heavy chain designed by the substitution of amino acid number 35 (arginine) with glycine, amino acid number 38 (lysine) with arginine, amino acid number 42 (leucine) with alanine, amino acid number 94 (alanine) with serine, amino acid number 95 (arginine) with lysine, amino acid number 103 (threonine) with asparagine, amino acid number 107 (serine) with alanine, and amino acid number 135 (valine) with leucine in the heavy chain of chimeric cA2-27D shown in SEQ ID NO: 24 was designated as "humanized hA2-27D-H4 type heavy chain" (sometimes also referred to as "hA2-27D-H4").

[0312]

The amino acid sequence of the humanized hA2-27D-H4 type heavy chain is described in SEQ ID NO: 46 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 19, a sequence consisting of amino acid numbers 20 to 140, and a sequence consisting of amino acid numbers 141 to 470 in the amino acid sequence of SEQ ID NO: 46 correspond to

the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 46 is described in SEQ ID NO: 45 in the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 57, a sequence consisting of nucleotide numbers 58 to 420, and a sequence consisting of nucleotide numbers 421 to 1410 in the nucleotide sequence of SEQ ID NO: 45 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 45 and the amino acid sequence of SEQ ID NO: 46 are also described in Figure 24.

[0313]

7)-6-5 Humanized hA2-27D-H5 type heavy chain

A humanized hA2-27D heavy chain designed by the substitution of amino acid number 35 (arginine) with glycine, amino acid number 38 (lysine) with arginine, amino acid number 42 (leucine) with alanine, amino acid number 95 (arginine) with lysine, amino acid number 103 (threonine) with asparagine, and amino acid number 135 (valine) with leucine in the heavy chain of chimeric cA2-27D shown in SEQ ID NO: 24 was designated as "humanized hA2-27D-H5 type heavy chain" (sometimes also referred to as "hA2-27D-H5").

[0314]

The amino acid sequence of the humanized hA2-27D-H5 type heavy chain is described in SEQ ID NO: 48 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 19, a sequence consisting of amino acid numbers 20 to 140, and a sequence consisting of amino acid numbers 141 to 470 in the amino acid sequence of SEQ ID NO: 48 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 48 is described in SEQ ID NO: 47 in the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 57, a sequence consisting of nucleotide numbers 58 to 420, and a sequence consisting of nucleotide numbers 421 to 1410 in the nucleotide sequence of SEQ ID NO: 47 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 47 and the amino acid sequence of SEQ ID NO: 48 are also described in Figure 25.

[0315]

7)-7 Humanization of A2-27D light chains

7)-7-1 Humanized hA2-27D-L1 type light chain

A humanized hA2-27D light chain designed by the substitution of amino acid number 29 (threonine) with glycine, amino acid number 31 (methionine) with leucine, amino acid number 32 (alanine) with serine, amino acid number 33 (alanine) with leucine, amino acid number 38 (lysine) with arginine, amino acid number 39 (valine) with alanine, amino acid number 42 (asparagine) with serine, amino acid number 59 (serine) with proline, amino acid number 61 (alanine) with glutamine, amino acid number 62 (serine) with alanine, amino acid number 64 (lysine) with arginine, amino acid number 66 (tryptophan) with leucine, amino acid number 77 (valine) with isoleucine, amino acid number 79 (asparagine) with aspartic acid, amino acid number 89 (serine) with aspartic acid, amino acid number 90 (tyrosine) with phenylalanine, amino acid number 91 (serine) with threonine, amino acid number 93 (alanine) with threonine, amino acid number 96 (serine) with arginine, amino acid number 97 (methionine) with leucine, amino acid number 99 (alanine) with proline, amino acid number 102 (valine) with phenylalanine, amino acid number 104 (threonine) with valine, amino acid number 120 (alanine) with glutamine, amino acid number 124 (leucine) with valine, and amino acid number 126 (leucine) with isoleucine in the light chain of chimeric cA2-27D shown in SEQ ID NO: 26 was designated as "humanized hA2-27D-L1 type light chain" (sometimes also referred to as "hA2-27D-L1").

[0316]

The amino acid sequence of the humanized hA2-27D-L1 type light chain is described in SEQ ID NO: 50 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20, a sequence consisting of amino acid numbers 21 to 129, and a sequence consisting of amino acid numbers 130 to 234 in the amino acid sequence of SEQ ID NO: 50 correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 50 is described in SEQ ID NO: 49 in the Sequence Listing. A sequence consisting of nucleotide numbers 26 to 85, a sequence consisting of nucleotide numbers 86 to 412, and a sequence

consisting of nucleotide numbers 413 to 727 in the nucleotide sequence of SEQ ID NO: 49 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 49 and the amino acid sequence of SEQ ID NO: 50 are also described in Figure 26.

[0317]

7)-7-2 Humanized hA2-27D-L2 type light chain

A humanized hA2-27D light chain designed by the substitution of amino acid number 29 (threonine) with glycine, amino acid number 31 (methionine) with leucine, amino acid number 32 (alanine) with serine, amino acid number 33 (alanine) with leucine, amino acid number 38 (lysine) with arginine, amino acid number 39 (valine) with alanine, amino acid number 42 (asparagine) with serine, amino acid number 59 (serine) with proline, amino acid number 61 (alanine) with glutamine, amino acid number 64 (lysine) with arginine, amino acid number 79 (asparagine) with aspartic acid, amino acid number 89 (serine) with aspartic acid, amino acid number 90 (tyrosine) with phenylalanine, amino acid number 91 (serine) with threonine, amino acid number 93 (alanine) with threonine, amino acid number 96 (serine) with arginine, amino acid number 97 (methionine) with leucine, amino acid number 99 (alanine) with proline, amino acid number 102 (valine) with phenylalanine, amino acid number 104 (threonine) with valine, amino acid number 120 (alanine) with glutamine, amino acid number 124 (leucine) with valine, and amino acid number 126 (leucine) with isoleucine in the light chain of chimeric cA2-27D shown in SEQ ID NO: 26 was designated as "humanized hA2-27D-L2 type light chain" (sometimes also referred to as "hA2-27D-L2").

[0318]

The amino acid sequence of the humanized hA2-27D-L2 type light chain is described in SEQ ID NO: 52 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20, a sequence consisting of amino acid numbers 21 to 129, and a sequence consisting of amino acid numbers 130 to 234 in the amino acid sequence of SEQ ID NO: 52 correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 52 is described in SEQ ID NO: 51 in the Sequence Listing. A sequence consisting of nucleotide

numbers 26 to 85, a sequence consisting of nucleotide numbers 86 to 412, and a sequence consisting of nucleotide numbers 413 to 727 in the nucleotide sequence of SEQ ID NO: 51 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 51 and the amino acid sequence of SEQ ID NO: 52 are also described in Figure 27.

[0319]

7)-7-3 Humanized hA2-27D-L3 type light chain

A humanized hA2-27D light chain designed by the substitution of amino acid number 29 (threonine) with glycine, amino acid number 31 (methionine) with leucine, amino acid number 32 (alanine) with serine, amino acid number 33 (alanine) with leucine, amino acid number 38 (lysine) with arginine, amino acid number 39 (valine) with alanine, amino acid number 42 (asparagine) with serine, amino acid number 59 (serine) with proline, amino acid number 64 (lysine) with arginine, amino acid number 79 (asparagine) with aspartic acid, amino acid number 89 (serine) with aspartic acid, amino acid number 91 (serine) with threonine, amino acid number 93 (alanine) with threonine, amino acid number 96 (serine) with arginine, amino acid number 97 (methionine) with leucine, amino acid number 99 (alanine) with proline, amino acid number 102 (valine) with phenylalanine, amino acid number 124 (leucine) with valine, and amino acid number 126 (leucine) with isoleucine in the light chain of chimeric cA2-27D shown in SEQ ID NO: 26 was designated as "humanized hA2-27D-L3 type light chain" (sometimes also referred to as "hA2-27D-L3").

[0320]

The amino acid sequence of the humanized hA2-27D-L3 type light chain is described in SEQ ID NO: 54 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20, a sequence consisting of amino acid numbers 21 to 129, and a sequence consisting of amino acid numbers 130 to 234 in the amino acid sequence of SEQ ID NO: 54 correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 54 is described in SEQ ID NO: 53 in the Sequence Listing. A sequence consisting of nucleotide numbers 26 to 85, a sequence consisting of nucleotide numbers 86 to 412, and a sequence

consisting of nucleotide numbers 413 to 727 in the nucleotide sequence of SEQ ID NO: 53 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 53 and the amino acid sequence of SEQ ID NO: 54 are also described in Figure 28.

[0321]

7)-7-4 Humanized hA2-27D-L4 type light chain

A humanized hA2-27D light chain was designed by the substitution of amino acid number 29 (threonine) with glycine, amino acid number 32 (alanine) with serine, amino acid number 38 (lysine) with arginine, amino acid number 42 (asparagine) with serine, amino acid number 59 (serine) with proline, amino acid number 64 (lysine) with arginine, amino acid number 79 (asparagine) with aspartic acid, amino acid number 89 (serine) with aspartic acid, amino acid number 91 (serine) with threonine, amino acid number 93 (alanine) with threonine, amino acid number 96 (serine) with arginine, amino acid number 99 (alanine) with proline, and amino acid number 102 (valine) with phenylalanine in the light chain of chimeric cA2-27D shown in SEQ ID NO: 26 was designated as "humanized hA2-27D-L4 type light chain" (sometimes also referred to as "hA2-27D-L4").

[0322]

The amino acid sequence of the humanized hA2-27D-L4 type light chain is described in SEQ ID NO: 56 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20, a sequence consisting of amino acid numbers 21 to 129, and a sequence consisting of amino acid numbers 130 to 234 in the amino acid sequence of SEQ ID NO: 56 correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 56 is described in SEQ ID NO: 55 in the Sequence Listing. A sequence consisting of nucleotide numbers 26 to 85, a sequence consisting of nucleotide numbers 86 to 412, and a sequence consisting of nucleotide numbers 413 to 727 in the nucleotide sequence of SEQ ID NO: 55 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 55 and the amino acid sequence of SEQ ID NO: 56 are also described in Figure 29.

[0323]

7)-7-5 Humanized hA2-27D-L5 type light chain

A humanized hA2-27D light chain designed by the substitution of amino acid number 29 (threonine) with glycine, amino acid number 32 (alanine) with serine, amino acid number 38 (lysine) with arginine, amino acid number 91 (serine) with threonine, amino acid number 93 (alanine) with threonine, amino acid number 96 (serine) with arginine, amino acid number 99 (alanine) with proline, and amino acid number 102 (valine) with phenylalanine in the light chain of chimeric cA2-27D shown in SEQ ID NO: 26 was designated as "humanized hA2-27D-L5 type light chain" (sometimes also referred to as "hA2-27D-L5").

[0324]

The amino acid sequence of the humanized hA2-27D-L5 type light chain is described in SEQ ID NO: 58 in the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20, a sequence consisting of amino acid numbers 21 to 129, and a sequence consisting of amino acid numbers 130 to 234 in the amino acid sequence of SEQ ID NO: 58 correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 58 is described in SEQ ID NO: 57 in the Sequence Listing. A sequence consisting of nucleotide numbers 26 to 85, a sequence consisting of nucleotide numbers 86 to 412, and a sequence consisting of nucleotide numbers 413 to 727 in the nucleotide sequence of SEQ ID NO: 57 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 57 and the amino acid sequence of SEQ ID NO: 58 are also described in Figure 30.

[0325]

7)-8 Design of humanized hA2-27D by combinations of heavy chains and light chains

An antibody consisting of the humanized hA2-27D-H1 type heavy chain and the humanized hA2-27D-L1 type light chain was designed and designated as "humanized hA2-27D-H1/L1" (sometimes also referred to as "hA2-27D-H1/L1"). An antibody consisting of the humanized hA2-27D-H1 type heavy chain and the humanized hA2-27D-L2 type light chain was designed and designated as "humanized hA2-27D-H1/L2" (sometimes also referred

to as "hA2-27D-H1/L2"). An antibody consisting of the humanized hA2-27D-H1 type heavy chain and the humanized hA2-27D-L3 type light chain was designed and designated as "humanized hA2-27D-H1/L3" (sometimes also referred to as "hA2-27D-H1/L3"). An antibody consisting of the humanized hA2-27D-H2 type heavy chain and the humanized hA2-27D-L1 type light chain was designed and designated as "humanized hA2-27D-H2/L1" (sometimes also referred to as "hA2-27D-H2/L1"). An antibody consisting of the humanized hA2-27D-H2 type heavy chain and the humanized hA2-27D-L2 type light chain was designed and designated as "humanized hA2-27D-H2/L2" (sometimes also referred to as "hA2-27D-H2/L2"). An antibody consisting of the humanized hA2-27D-H2 type heavy chain and the humanized hA2-27D-L3 type light chain was designed and designated as "humanized hA2-27D-H2/L3" (sometimes also referred to as "hA2-27D-H2/L3"). An antibody consisting of the humanized hA2-27D-H3 type heavy chain and the humanized hA2-27D-L1 type light chain was designed and designated as "humanized hA2-27D-H3/L1" (sometimes also referred to as "hA2-27D-H3/L1"). An antibody consisting of the humanized hA2-27D-H3 type heavy chain and the humanized hA2-27D-L2 type light chain was designed and designated as "humanized hA2-27D-H3/L2" (sometimes also referred to as "hA2-27D-H3/L2"). An antibody consisting of the humanized hA2-27D-H3 type heavy chain and the humanized hA2-27D-L3 type light chain was designed and designated as "humanized hA2-27D-H3/L3" (sometimes also referred to as "hA2-27D-H3/L3"). An antibody consisting of the humanized hA2-27D-H3 type heavy chain and the humanized hA2-27D-L4 type light chain was designed and designated as "humanized hA2-27D-H3/L4" (sometimes also referred to as "hA2-27D-H3/L4"). An antibody consisting of the humanized hA2-27D-H4 type heavy chain and the humanized hA2-27D-L3 type light chain was designed and designated as "humanized hA2-27D-H4/L3" (sometimes also referred to as "hA2-27D-H4/L3"). An antibody consisting of the humanized hA2-27D-H4 type heavy chain and the humanized hA2-27D-L4 type light chain was designed and designated as "humanized hA2-27D-H4/L4" (sometimes also referred to as "hA2-27D-H4/L4"). An antibody consisting of the humanized hA2-27D-H4 type heavy chain and the humanized hA2-27D-L5 type light chain was designed and designated as "humanized hA2-27D-H4/L5" (sometimes also referred to as "hA2-27D-H4/L5"). An

antibody consisting of the humanized hA2-27D-H5 type heavy chain and the humanized hA2-27D-L4 type light chain was designed and designated as "humanized hA2-27D-H5/L4" (sometimes also referred to as "hA2-27D-H5/L4"). The antibodies designed as above can be prepared in accordance with Example 8, and evaluated in accordance with Examples 2 and 4.

[0326]

<Example 8>

Construction of humanized A2-15A antibody and humanized A2-27D antibody expression vectors and preparation of the antibodies

8)-1 Construction of humanized A2-15A heavy chain expression vector

8)-1-1 Construction of humanized hA2-15A-H1 type heavy chain expression vector

A DNA fragment containing a humanized hA2-15A-H1 variable region-encoding DNA sequence represented by nucleotide numbers 36 to 443 of the nucleotide sequence of humanized hA2-15A-H1 shown in SEQ ID NO: 27 was synthesized (GeneArt Artificial Gene Synthesis Service). The DNA fragment containing a humanized hA2-15A-H1 variable region-encoding DNA sequence was amplified using the synthesized DNA fragment as a template, KOD-Plus- (Toyobo Co., Ltd., Japan), and a primer set given below, and inserted at the restriction enzyme BspI-cleaved site of the chimeric and humanized antibody IgG1 type heavy chain expression vector pCMA-G1 using In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.) to construct a humanized hA2-15A-H1 expression vector. The obtained expression vector was designated as "pCMA-G1/hA2-15A-H1".

Primer set

5'-AGCTCCCAGATGGGTGCTGAGC-3' (EG-Inf-F; SEQ ID NO: 99)

5'-GGGCCCTTGGTGGAGGCTGAGC-3' (EG1-Inf-R; SEQ ID NO: 100)

[0327]

8)-1-2 Construction of humanized hA2-15A-H4 type heavy chain expression vector

A DNA fragment containing a humanized hA2-15A-H4 variable region-encoding DNA sequence represented by nucleotide numbers 36 to 443 of the nucleotide sequence of humanized hA2-15A-H4 shown in SEQ ID NO: 29 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-15A-H4 expression vector was constructed in the

same way as in Example 8)-1-1. The obtained expression vector was designated as "pCMA-G1/hA2-15A-H4".

[0328]

8)-2 Construction of humanized A2-15A light chain expression vector

8)-2-1 Construction of humanized hA2-15A-L1 type light chain expression vector

A DNA fragment containing a sequence encoding humanized hA2-15A-L1, of SEQ ID NO: 31, was synthesized (GeneArt Gene Synthesis Service). The DNA fragment containing a sequence encoding humanized hA2-15A-L1 was amplified using the synthesized DNA fragment as a template, KOD-Plus- (Toyobo Co., Ltd.), and a primer set given below, and inserted at the site from which the sequence encoding a κ chain secretory signal and a human κ chain constant region was removed by the digestion of the expression vector pCMA-LK with restriction enzymes XbaI and PmeI, using In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.) to construct a humanized hA2-15A-L1 expression vector. The obtained expression vector was designated as "pCMA/hA2-15A-L1".

Primer set

5'-CCAGCCTCCGGACTCTAGAGCCACC-3' (CM-inf-F; SEQ ID NO: 101)

5'-AGTTAGCCTCCCCGTTTAAACTC-3' (CM-inf-R; SEQ ID NO: 102)

[0329]

8)-2-2 Construction of humanized hA2-15A-L4 type light chain expression vector

A DNA fragment containing a sequence encoding humanized hA2-15A-L4, of SEQ ID NO: 33, was synthesized (GeneArt Gene Synthesis Service). A humanized hA2-15A-L4 expression vector was constructed in the same way as in Example 8)-2-1. The obtained expression vector was designated as "pCMA/hA2-15A-L4".

[0330]

8)-2-3 Construction of humanized hA2-15A-L6 type light chain expression vector

A DNA fragment containing a sequence encoding humanized hA2-15A-L6, as shown in SEQ ID NO: 35, was synthesized (GeneArt Gene Synthesis Service). A humanized hA2-15A-L6 expression vector was constructed in the same way as in Example 8)-2-1. The obtained expression vector was designated as "pCMA/hA2-15A-L6".

[0331]

8)-2-4 Construction of humanized hA2-15A-L7 type light chain expression vector

A DNA fragment containing a sequence encoding humanized hA2-15A-L7, as shown in SEQ ID NO: 37, was synthesized (GeneArt Gene Synthesis Service). A humanized hA2-15A-L7 expression vector was constructed in the same way as in Example 8)-2-1. The obtained expression vector was designated as "pCMA/hA2-15A-L7".

[0332]

8)-3 Construction of humanized A2-27D heavy chain expression vector

8)-3-1 Construction of humanized hA2-27D-H1 type heavy chain expression vector

A DNA fragment containing a humanized hA2-27D-H1 variable region-encoding DNA sequence represented by nucleotide numbers 36 to 437 of the nucleotide sequence of humanized hA2-27D-H1 of SEQ ID NO: 39 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-27D-H1 expression vector was constructed in the same way as in Example 8)-1-1. The obtained expression vector was designated as "pCMA-G1/hA2-27D-H1".

[0333]

8)-3-2 Construction of humanized hA2-27D-H2 type heavy chain expression vector

A DNA fragment containing a humanized hA2-27D-H2 variable region-encoding DNA sequence represented by nucleotide numbers 36 to 437 of the nucleotide sequence of humanized hA2-27D-H2 of SEQ ID NO: 41 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-27D-H2 expression vector was constructed in the same way as in Example 8)-1-1. The obtained expression vector was designated as "pCMA-G1/hA2-27D-H2".

[0334]

8)-3-3 Construction of humanized hA2-27D-H3 type heavy chain expression vector

A DNA fragment containing a humanized hA2-27D-H3 variable region-encoding DNA sequence represented by nucleotide numbers 36 to 437 of the nucleotide sequence of humanized hA2-27D-H3 of SEQ ID NO: 43 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-27D-H3 expression vector was constructed in the

same way as in Example 8)-1-1. The obtained expression vector was designated as "pCMA-G1/hA2-27D-H3".

[0335]

8)-3-4 Construction of humanized hA2-27D-H4 type heavy chain expression vector

A DNA fragment containing a humanized hA2-27D-H4 variable region-encoding DNA sequence represented by nucleotide numbers 36 to 437 of the nucleotide sequence of humanized hA2-27D-H4 of SEQ ID NO: 45 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-27D-H4 expression vector was constructed in the same way as in Example 8)-1-1. The obtained expression vector was designated as "pCMA-G1/hA2-27D-H4".

[0336]

8)-3-5 Construction of humanized hA2-27D-H5 type heavy chain expression vector

A DNA fragment containing a humanized hA2-27D-H5 variable region-encoding DNA sequence represented by nucleotide numbers 36 to 437 of the nucleotide sequence of humanized hA2-27D-H5 of SEQ ID NO: 47 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-27D-H5 expression vector was constructed in the same way as in Example 8)-1-1. The obtained expression vector was designated as "pCMA-G1/hA2-27D-H5".

[0337]

8)-4 Construction of humanized A2-27D light chain expression vector

8)-4-1 Construction of humanized hA2-27D-L1 type light chain expression vector

A DNA fragment containing a sequence encoding humanized hA2-27D-L1, as shown in SEQ ID NO: 49, was synthesized (GeneArt Gene Synthesis Service). A humanized hA2-27D-L1 expression vector was constructed in the same way as in Example 8)-2-1. The obtained expression vector was designated as "pCMA/hA2-27D-L1".

[0338]

8)-4-2 Construction of humanized hA2-27D-L2 type light chain expression vector

A DNA fragment containing a sequence encoding humanized hA2-27D-L2, as shown in SEQ ID NO: 51, was synthesized (GeneArt Gene Synthesis Service). A humanized hA2-

27D-L2 expression vector was constructed in the same way as in Example 8)-2-1. The obtained expression vector was designated as "pCMA/hA2-27D-L2".

[0339]

8)-4-3 Construction of humanized hA2-27D-L3 type light chain expression vector

A DNA fragment containing a sequence encoding humanized hA2-27D-L3, as shown in SEQ ID NO: 53, was synthesized (GeneArt Gene Synthesis Service). A humanized hA2-27D-L3 expression vector was constructed in the same way as in Example 8)-2-1. The obtained expression vector was designated as "pCMA/hA2-27D-L3".

[0340]

8)-4-4 Construction of humanized hA2-27D-L4 type light chain expression vector

A DNA fragment containing a sequence encoding humanized hA2-27D-L4, as shown in SEQ ID NO: 55, was synthesized (GeneArt Gene Synthesis Service). A humanized hA2-27D-L4 expression vector was constructed in the same way as in Example 8)-2-1. The obtained expression vector was designated as "pCMA/hA2-27D-L4".

[0341]

8)-4-5 Construction of humanized hA2-27D-L5 type light chain expression vector

A DNA fragment containing a sequence encoding humanized hA2-27D-L5, as shown in SEQ ID NO: 57, was synthesized (GeneArt Gene Synthesis Service). A humanized hA2-27D-L5 expression vector was constructed in the same way as in Example 8)-2-1. The obtained expression vector was designated as "pCMA/hA2-27D-L5".

[0342]

8)-5 Preparation of humanized A2-15A antibody (IgG1) and humanized A2-27D antibody (IgG1)

8)-5-1 Production of humanized A2-15A antibody (IgG1) and humanized A2-27D antibody (IgG1)

Each antibody was obtained in the same way as in Example 5)-7. Specifically, humanized hA2-15A-H4/L6 was obtained by the combination of pCMA-G1/hA2-15A-H4 constructed in Example 8)-1-2 and pCMA/hA2-15A-L6 constructed in Example 8)-2-3. Humanized hA2-27D-H2/L2 was obtained by the combination of pCMA-G1/hA2-27D-H2

constructed in Example 8)-3-2 and pCMA/hA2-27D-L2 constructed in Example 8)-4-2. Humanized hA2-27D-H3/L4 was obtained by the combination of pCMA-G1/hA2-27D-H3 constructed in Example 8)-3-3 and pCMA/hA2-27D-L4 constructed in Example 8)-4-4.

[0343]

8)-5-2 Two-step purification of humanized A2-15A antibody (IgG1) and humanized A2-27D antibody (IgG1)

Each culture supernatant obtained in Example 8)-5-1 was purified in the same way as in Example 5)-8.

[0344]

<Example 9>

In vitro activity evaluation of humanized A2-15A antibody (IgG1) and humanized A2-27D antibody (IgG1)

9)-1 Antibody evaluation by luciferase reporter assay

Humanized hA2-15A-H4/L6, humanized hA2-27D-H2/L2, and humanized hA2-27D-H3/L4 prepared in Example 8)-5 were analyzed for their inhibitory activity against ALK2-mediated intracellular signals using a luciferase reporter specific for BMP. HEPG2 cells were transfected with pGL4.26/Id1WT4F-luc (Genes Cells, 7, 949 (2002)) in the same way as in Example 6)-1. After 3 hours, the medium was replaced with fresh FreeStyle 293 expression medium (Invitrogen Corp.). Then, each humanized antibody and 10 ng/mL BMP7 (manufactured by Miltenyi Biotec) were added thereto. On the next day, luciferase activity was measured.

[0345]

The results are shown in Figure 31. Humanized hA2-15A-H4/L6, humanized hA2-27D-H2/L2, and humanized hA2-27D-H3/L4 were confirmed to inhibit, in a dose-dependent manner, BMP-specific luciferase activity induced by BMP7.

[0346]

<Example 10>

Evaluation of binding activity of humanized A2-15A antibody (IgG1) and humanized A2-27D antibody (IgG1) against human ALK2

The dissociation constants of humanized hA2-15A-H4/L6, humanized hA2-27D-H2/L2, and humanized hA2-27D-H3/L4 prepared in Example 8)-5 for the antigen (Recombinant Human ALK2 Fc Chimera, Sino Biological Inc.) were measured in Biacore T200 (GE Healthcare Bio-Sciences Corp.) by using the antigen immobilized as a ligand and each antibody as an analyte. The antigen was added at 1.25 μg/mL for 60 seconds and immobilized onto a sensor chip CM5 (GE Healthcare Bio-Sciences Corp.). The running buffer used was HBS-EP+ (10 mM HEPES (pH 7.4), 0.15 M NaCl, 3 mM EDTA, and 0.05% Surfactant P20). Serially diluted solutions (0.2 to 50 nM) of the antibody were added onto the antigen-immobilized chip at a flow rate of 30 μl/min for 300 seconds. Subsequently, the dissociation phase was monitored for 1800 seconds. A 10 mM glycine/hydrochloric acid solution (pH 1.5) was added thereto as a regenerating solution at a flow rate of 10 μl/min for 30 seconds twice. The data was analyzed using bivalent binding models of analytical software (BIAevaluation software, version 1.0) to calculate an association rate constant k_a , a dissociation rate constant k_d , and a dissociation constant (K_D ; $K_D = k_d/k_a$).

[0347]

The results are shown in Table 1.

[Table 1]

Dissociation constants of humanized A2-15A antibody and humanized A2-27D antibody

	Name	KD(nM)
1	hA2-27D-H2/L2	5.7
2	hA2-27D-H3/L4	4.3
3	hA2-15A-H4/L6	4.3

[0348]

<Example 11>

Preparation of humanized A2-15A antibody (IgG2)

11)-1 Construction of humanized IgG2 type heavy chain expression vector pCMA-G2
pCMA-LK constructed in Example 5)-1 was digested with XbaI and PmeI. The obtained DNA fragment except for the DNA sequence encoding the κ chain secretory signal

and the human κ chain constant region was ligated with a DNA fragment (SEQ ID NO: 103) containing a nucleotide sequence encoding the amino acid sequence of a human heavy chain secretory signal sequence and a human IgG2 constant region using In-Fusion Advantage PCR cloning kit (Clontech Laboratories, Inc.) to construct a chimeric and humanized IgG2 type heavy chain expression vector pCMA-G2 having a signal sequence, a cloning site, and the nucleotide sequence encoding the human IgG2 heavy chain constant region, downstream of the CMV promoter.

[0349]

11)-2 Construction of humanized hA2-15A-H4 IgG2 type heavy chain expression vector

A DNA fragment containing heavy chain variable region-encoding cDNA was amplified using pCMA-G1/hA2-15A-H4 constructed in Example 8)-1-2 as a template, KOD-Plus- (Toyobo Co., Ltd.), and a primer set given below, and inserted at the restriction enzyme BlnI-cleaved site of the humanized IgG2 type heavy chain expression vector pCMA-G2 using In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.) to construct a humanized hA2-15A-H4 IgG2 type expression vector. The obtained expression vector was designated as "pCMA-G2/hA2-15A-H4".

[0350]

The nucleotide sequence of the humanized hA2-15A-H4 IgG2 type heavy chain is shown in SEQ ID NO: 104, and the amino acid sequence thereof is shown in SEQ ID NO: 105. A sequence consisting of nucleotide numbers 1 to 57 of the nucleotide sequence of SEQ ID NO: 104, a sequence consisting of nucleotide numbers 58 to 426 thereof, and a sequence consisting of nucleotide numbers 427 to 1404 thereof encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. A sequence consisting of amino acid numbers 1 to 19 of the amino acid sequence of SEQ ID NO: 105, a sequence consisting of amino acid numbers 20 to 142 thereof, and a sequence consisting of amino acid numbers 143 to 468 thereof correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The

nucleotide sequence of SEQ ID NO: 104 and the amino acid sequence of SEQ ID NO: 105 are also described in Figure 32.

Primer set for humanized hA2-15A-H4 IgG2 type heavy chain

5'-CAGATGGGTGCTGAGCGAAGTGCAGCTGGTGGGAATCTGGC-3' (15A-H4-F; SEQ ID NO: 130)

5'-CTTGGTGCTGGCTGAGCTGACGGTCACGAGGGTGCC-3' (15A-H4-R; SEQ ID NO: 131)

[0351]

11)-3 Production of humanized A2-15A antibody(IgG2)

The antibody was produced in the same way as in Example 5)-7. A humanized A2-15A antibody obtained by the combination of pCMA-G2/hA2-15A-H4 constructed in Example 11)-2 and pCMA/hA2-15A-L6 constructed Example 8)-2-3 was designated as "humanized hA2-15A-H4/L6 (IgG2)".

11)-4 Two-step purification of humanized hA2-15A-H4/L6 (IgG2)

The culture supernatant obtained in Example 11)-3 was purified in the same way as in Example 5)-8.

[0352]

<Example 12>

Preparation of humanized A2-27D antibody (IgG1 LALA)

12)-1 Construction of humanized hA2-27D-H2-LALA type heavy chain expression vector

Mutations were introduced using pCMA-G1/hA2-27D-H2 constructed in Example 8)-3-2 as a template, a primer set given below, and KOD -Plus- Mutagenesis Kit (Toyobo Co., Ltd.) to construct a hA2-27D-H2-LALA type heavy chain expression vector. The constructed expression vector was designated as "pCMA-G1/hA2-27D-H2-LALA".

[0353]

The nucleotide sequence of the humanized hA2-27D-H2-LALA type heavy chain is shown in SEQ ID NO: 106, and the amino acid sequence thereof is shown in SEQ ID NO: 107. A sequence consisting of nucleotide numbers 1 to 57 of the nucleotide sequence of SEQ ID

NO: 106, a sequence consisting of nucleotide numbers 58 to 420 thereof, and a sequence consisting of nucleotide numbers 421 to 1410 thereof encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. A sequence consisting of amino acid numbers 1 to 19 of the amino acid sequence of SEQ ID NO: 107, a sequence consisting of amino acid numbers 20 to 140 thereof, and a sequence consisting of amino acid numbers 141 to 470 thereof correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence of SEQ ID NO: 106 and the amino acid sequence of SEQ ID NO: 107 are also described in Figure 33.

Primer set:

5'-GCGGGGGGACCCTCAGTCTTCCTCTTCCCC-3' (LALA-F; SEQ ID NO: 132)

5'-GGCTTCAGGTGCTGGGCAGGGTGGGCATGTG-3' (LALA-R; SEQ ID NO: 133)

[0354]

12)-2 Construction of humanized hA2-27D-H3-LALA type heavy chain expression vector

A hA2-27D-H3-LALA type heavy chain expression vector was constructed in the same way as in Example 12)-1 using pCMA-G1/hA2-27D-H3 constructed in Example 8)-3-3 as a template. The constructed expression vector was designated as "pCMA-G1/hA2-27D-H3-LALA".

[0355]

The nucleotide sequence of the humanized hA2-27D-H3-LALA type heavy chain is shown in SEQ ID NO: 108, and the amino acid sequence thereof is shown in SEQ ID NO: 109. A sequence consisting of nucleotide numbers 1 to 57 of the nucleotide sequence of SEQ ID NO: 108, a sequence consisting of nucleotide numbers 58 to 420 thereof, and a sequence consisting of nucleotide numbers 421 to 1410 thereof encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. A sequence consisting of amino acid numbers 1 to 19 of the amino acid sequence of SEQ ID NO: 109, a sequence consisting of amino acid numbers 20 to 140 thereof, and a sequence consisting of amino acid numbers 141 to 470 thereof correspond to the signal sequence, the

heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence of SEQ ID NO: 108 and the amino acid sequence of SEQ ID NO: 109 are also described in Figure 34.

[0356]

12)-3 Production of humanized A2-27D antibody (IgG1 LALA)

Each antibody was produced in the same way as in Example 5)-7. A humanized A2-27D antibody obtained by the combination of pCMA-G1/hA2-27D-H2-LALA constructed in Example 12)-1 and pCMA/hA2-27D-L2 constructed in Example 8)-4-2 was designated as "humanized hA2-27D-H2/L2 (IgG1 LALA)". A humanized A2-27D antibody obtained by the combination of pCMA-G1/hA2-27D-H3-LALA constructed in Example 12)-2 and pCMA/hA2-27D-L4 constructed in Example 8)-4-4 was designated as "humanized hA2-27D-H3/L4 (IgG1 LALA)".

[0357]

12)-4 Two-step purification of humanized A2-27D antibody (IgG1 LALA)

Each culture supernatant obtained in Example 12)-3 was purified in the same way as in Example 5)-8.

[0358]

<Example 13>

In vitro activity evaluation of humanized A2-15A antibody (IgG2) and humanized A2-27D antibody (IgG1 LALA)

13)-1 Antibody evaluation by luciferase reporter assay

Humanized A2-15A-H4/L6 (IgG2) prepared in Example 11)-4 and humanized A2-27D-H2/L2 (IgG1 LALA) obtained in Example 12)-4 were analyzed for their inhibitory activity against ALK2-mediated intracellular signals using a luciferase reporter specific for BMP. HEPG2 cells were transfected with pGL4.26/Id1WT4F-luc (Genes Cells, 7, 949 (2002)) in the same way as in Example 6)-1. After 3 hours, the medium was replaced with fresh FreeStyle 293 expression medium (Invitrogen Corp.). Then, each humanized antibody and 2.5 ng/mL BMP9 were added thereto. On the next day, luciferase activity was measured in the same way as in Example 6)-1.

[0359]

The results are shown in Figure 35. Humanized A2-15A-H4/L6 (IgG2) and humanized A2-27D-H2/L2 (IgG1 LALA) were confirmed to inhibit, in a dose-dependent manner, BMP-specific luciferase activity induced by BMP7.

[0360]

<Example 14>

Evaluation of binding activity of humanized A2-15A antibody (IgG2) and humanized A2-27D antibody (IgG1 LALA) against human ALK2

14)-1 Expression and purification of human ALK2 extracellular domain

DNA encoding a human ALK2 extracellular domain (polypeptide consisting of amino acid numbers 21 to 123 of the amino acid sequence of Accession No. NP_001096 of the NCBI protein database) was inserted into a vector pET-28b(+) (Novagen/Merck KGaA, Catalog No: 69865) to construct a plasmid for the expression of a protein C-terminally linked to a His tag sequence. *E. coli* SHuffle T7 (New England Biolabs Inc., Catalog No: C3029H) was transformed with this plasmid and cultured in TB medium (Sigma-Aldrich Co. LLC, Catalog No: T0918). The *E. coli* thus cultured was ultrasonicated, and the obtained bacterial cells were centrifuged. The supernatant was purified using HisTrap FF crude column (GE Healthcare Bio-Sciences Corp., Catalog No: 17-5286-01). Then, the human ALK2 extracellular domain was purified by electrophoresis using HiLoad 26/600 Superdex 200 column (GE Healthcare Bio-Sciences Corp., Catalog No: 28-9893-36) until a single band with a molecular weight of 12 kDa was obtained.

[0361]

14)-2 Measurement of ability of humanized A2-15A antibody (IgG2) and humanized A2-27D antibody (IgG1 LALA) to bind to antigen

The dissociation constants of humanized hA2-15A-H4/L6 (IgG2) prepared in Example 11)-4, and humanized hA2-27D-H2/L2 (IgG1 LALA) and humanized hA2-27D-H3/L4 (IgG1 LALA) prepared in Example 12)-4 for the antigen (human ALK2 extracellular domain prepared in Example 14)-1) were measured using Biacore T200 (GE Healthcare Bio-Sciences Corp., Japan) by the capture method which involves capturing each antibody as a ligand onto

an immobilized anti-human IgG (Fc) antibody and assaying the antigen as an analyte. Approximately 1000 RU of the anti-human IgG (Fc) antibody (Human Antibody Capture kit, GE Healthcare Bio-Sciences Corp.) was covalently bound to a sensor chip CM5 (GE Healthcare Bio-Sciences Corp.) by the amine coupling method. Similarly, this antibody was immobilized onto a reference cell. The running buffer used was HBS-EP+ (10 mM HEPES (pH 7.4), 0.15 M NaCl, 3 mM EDTA, and 0.05% Surfactant P20). The antibody was added onto the anti-human IgG (Fc) antibody-immobilized chip for approximately 1 minute. Then, serially diluted solutions (0.78 to 200 nM) of the antigen were added thereto at a flow rate of 30 µl/min for 300 seconds. Subsequently, the dissociation phase was monitored for 600 seconds. A 3 M magnesium chloride solution was added thereto as a regenerating solution at a flow rate of 10 µl/min for 30 seconds. The data was analyzed using 1:1 binding models of analytical software (BIAevaluation software, version 1.0) to calculate an association rate constant k_a , a dissociation rate constant k_d , and a dissociation constant (K_D ; $K_D = k_d/k_a$).

[0362]

The results are shown in Table 2.

[Table 2]

Dissociation constants of humanized A2-15A antibody (IgG2) and humanized A2-27D antibody (IgG1 LALA)

	Name	KD(nM)
1	hA2-15A-H4/L6, IgG2	17.4
2	hA2-27D-H2/L2, IgG1 LALA	13.6
3	hA2-27D-H3/L4, IgG1 LALA	13.3

[0363]

<Example 15>

Evaluation of *in vivo* activity of humanized A2-15A antibody (IgG2) and humanized A2-27D antibody (IgG1 LALA)

Humanized hA2-15A-H4/L6 (IgG2) prepared in Example 11)-4 and humanized hA2-27D-H2/L2 (IgG1 LALA) prepared in Example 12)-4 were analyzed for their inhibitory activity against ectopic ossification using mouse models with ectopic ossification caused by

the transplantation of BMP7. CollaTape (manufactured by Zimmer Biomet Dental K.K.) cut into a round shape of 4 mm in diameter was impregnated with 2.5 µg of BMP7 (manufactured by Miltenyi Biotec), frozen overnight -80°C, and then dried in vacuum. Skin hair near the thigh bone of each mouse (C57BL/6; 8-9 weeks old) was shaved, and an incision was made in this area under anesthesia by the aspiration of isoflurane. The freeze-dried filter paper was transplanted into skeletal muscle. Two weeks after the transplantation, calcified ectopic bone was analyzed as ectopic ossification by micro-CT (manufactured by Comscantecno Co., Ltd.). Then, the ectopic bone in the skeletal muscle was isolated, and the weight was measured. Each antibody was subcutaneously administered once a week (days -1 and 6 when the transplantation day was defined as day 0). The antibody was diluted with a solvent (HBSor) such that its concentration was 1, 3, or 10 mg/kg. Control human IgG (manufactured by Jackson ImmunoResearch Laboratories, Inc.) was adjusted to 10 mg/kg with a solvent and administered to mice in a control group.

[0364]

The results are shown in Figure 36. Humanized hA2-15A-H4/L6 (IgG2) and humanized hA2-27D-H2/L2 (IgG1 LALA) were confirmed to suppress BMP7-induced ectopic osteoinduction in mouse skeletal muscle tissues.

[0365]

<Example 16>

Epitope analysis of A2-27D antibody

16)-1 Preparation of human chimeric cA2-27D Fab fragment

A Fab fragment was prepared from the human chimeric cA2-27D antibody obtained in Example 5)-8 using Pierce Fab Preparation Kit.

[0366]

16)-2 Crystallization and structural analysis of human chimeric cA2-27D Fab fragment and ALK2-ECD complex

The protein complex of the human chimeric cA2-27D Fab fragment obtained in Example 16)-1 and the ALK2-ECD prepared according to Example 14 were concentrated to 2.4 mg/mL and used in the crystallization trial employing vapor diffusion method. To 0.5 µL

of the protein solution, an equal amount of a precipitant solution (2% (v/v) Tacsimate (pH 7.0), 100 mM HEPES (pH 7.5), and 20% (w/v) polyethylene glycol 3,350) was added, and the resulting solution was placed in a sealed container containing 50 μ L of a precipitant solution such that these solutions had no contact with each other. The container was left standing at 20°C. Three days later, 0.1 mm $\times$ 0.1 mm $\times$ 0.1 mm single crystals were obtained. The obtained crystals were dipped in a precipitant solution supplemented with 20% (v/v) glycerol, and subsequently frozen in liquid nitrogen. X-ray diffraction data was collected under 95 K nitrogen stream using BL1A of KEK Photon Factory. Diffraction intensity was digitized from the obtained diffraction image using software XDS (Acta Cryst. (2010). D66, 125-132) to determine crystal structure factors. The crystals were in the body-centered monoclinic crystal system with a space group C121 and unit cells of $a = c = 119.39$ angstroms, $b = 37.32$ angstroms, and $\beta = 92.54$. The molecular replacement method was performed using the obtained structure factors and the three-dimensional structure coordinates of Fab (antibody structure determined by the past crystal structure analysis was used) to determine the phases. Software phaser (CCP4: Collaborative Computational Project No. 4) was used in calculation. The crystal contained one complex in the asymmetric unit. Structure refinement was performed using software Refmac5 (CCP4), and model correction was performed using software coot. This operation was repetitively performed to obtain a final R factor of 22.3% and a free R factor of 26.7% with a resolution of 2.6 angstroms. The model consists of one complex and contains amino acid numbers 1 to 211 of the A2-27D Fab L chain, amino acid numbers 1 to 134 and 141 to 223 of the A2-27D Fab H chain, amino acid numbers 11 to 89 of ALK2-ECD, and 61 water molecules. The determined amino acid residues of ALK2-ECD located within 4 angstroms from A2-27D Fab are as follows: Glu18, Gly19, Ile39, Asn40, Asp41, Gly42, Phe43, His44, Val45, Tyr46, Asn82, Thr84, Gln86, and Leu87. The ribbon model of the whole complex is shown in Figure 37.

[0367]

<Example 17>

Design of humanized A2-11E antibody and humanized A2-25C antibody

17)-1 Design of humanized hA2-11E

17)-1-1 Molecular modeling of A2-11E variable regions

The molecular modeling of the A2-11E variable regions was carried out by a method generally known as homology modeling (Methods in Enzymology, 203, 121-153, (1991)). The variable regions of A2-11E determined above were compared with 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)). As a result, 3BN9 was selected because of having the highest sequence identity to the heavy and light chain variable regions of A2-11E. The three-dimensional structures of framework regions were prepared as a "framework model" by combining the coordinates of 3BN9. Subsequently, the typical conformation of each CDR was incorporated into the framework model.

[0368]

Finally, energy minimization calculation for excluding disadvantageous interatomic contact was conducted in order to obtain possible molecular models of the A2-11E variable regions in terms of energy. These procedures were performed using a commercially available protein three-dimensional structure analysis program Discovery Studio (manufactured by Accelrys).

[0369]

17)-1-2 Design of amino acid sequence of humanized hA2-11E

Humanized hA2-11E was constructed by a method generally known as CDR grafting (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). An acceptor antibody was selected on the basis of the identity of amino acids in framework regions.

[0370]

The sequences of the framework regions of A2-11E were compared with the framework regions of human subgroup consensus sequences. As a result, a human γ chain subgroup 3 consensus sequence and a human κ chain subgroup 1 consensus sequence specified by KABAT et al. (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service National Institutes of Health, Bethesda, MD. (1991)) were selected as acceptors due to their high sequence identity as to framework regions. The amino acid

residues of the framework regions of the human γ chain subgroup 3 consensus sequence and the human κ chain subgroup 1 consensus sequence were aligned with the amino acid residues of the A2-11E framework regions to identify the numbers of amino acids that did not match therebetween. The numbers of these residues were analyzed using the three-dimensional model of A2-11E constructed above. Then, the donor residues to be grafted onto the acceptors were selected according to the criteria provided by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). Some donor residues thus selected were transferred to the acceptor antibody to construct the humanized hA2-11E sequence as described in Examples below.

[0371]

17)-2 Humanization of A2-11E heavy chain

17)-2-1 Humanized hA2-11E-H3 type heavy chain

A humanized hA2-11E heavy chain designed from the A2-11E heavy chain of SEQ ID NO: 2 of the Sequence Listing by the replacement of amino acid position 7 (threonine) with serine, amino acid position 16 (arginine) with glycine, amino acid position 19 (lysine) with arginine, amino acid position 23 (valine) with alanine, amino acid position 48 (isoleucine) with valine, amino acid position 61 (proline) with alanine, amino acid position 69 (alanine) with threonine, amino acid position 75 (alanine) with serine, amino acid position 76 (glutamic acid) with lysine, amino acid position 88 (serine) with alanine, and amino acid position 93 (threonine) with valine was designated as "hA2-11E-H3 type heavy chain".

[0372]

The amino acid sequence of the humanized hA2-11E-H3 type heavy chain is described in SEQ ID NO: 111 of the Sequence Listing. A sequence consisting of amino acid numbers 1 to 19 of the amino acid sequence of SEQ ID NO: 111, a sequence consisting of amino acid numbers 20 to 137 thereof, and a sequence consisting of amino acid numbers 138 to 467 thereof correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 111 is described in SEQ ID NO: 110 of the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 57 of the nucleotide sequence of SEQ ID NO:

110, a sequence consisting of nucleotide numbers 58 to 411 thereof, and a sequence consisting of nucleotide numbers 412 to 1401 thereof encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 110 and the amino acid sequence of SEQ ID NO: 111 are also described in Figure 38.

[0373]

17)-2-2 Humanized hA2-11E-H4 type heavy chain

A humanized hA2-11E heavy chain designed from the A2-11E heavy chain of SEQ ID NO: 2 of the Sequence Listing by the replacement of amino acid position 7 (threonine) with serine, amino acid position 16 (arginine) with glycine, amino acid position 19 (lysine) with arginine, amino acid position 23 (valine) with alanine, amino acid position 69 (alanine) with threonine, amino acid position 75 (alanine) with serine, amino acid position 76 (glutamic acid) with lysine, amino acid position 88 (serine) with alanine, and amino acid position 93 (threonine) with valine was designated as "hA2-11E-H4 type heavy chain".

[0374]

The amino acid sequence of the humanized hA2-11E-H4 type heavy chain is described in SEQ ID NO: 113 of the Sequence Listing. A sequence consisting of amino acid numbers 1 to 19 of the amino acid sequence of SEQ ID NO: 113, a sequence consisting of amino acid numbers 20 to 137 thereof, and a sequence consisting of amino acid numbers 138 to 467 thereof correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 113 is described in SEQ ID NO: 112 of the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 57 of the nucleotide sequence of SEQ ID NO: 112, a sequence consisting of nucleotide numbers 58 to 411 thereof, and a sequence consisting of nucleotide numbers 412 to 1401 thereof encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 112 and the amino acid sequence of SEQ ID NO: 113 are also described in Figure 39.

[0375]

17)-3 Humanization of A2-11E light chain

17)-3-1 Humanized hA2-11E-L2 type light chain

A humanized hA2-11E light chain designed from the A2-11E light chain of SEQ ID NO: 4 of the Sequence Listing by the replacement of amino acid number 10 (leucine) with serine, amino acid position 21 (leucine) with isoleucine, amino acid number 22 (serine) with threonine, amino acid number 40 (leucine) with proline, amino acid number 42 (glutamic acid) with lysine, amino acid number 58 (isoleucine) with valine, amino acid number 83 (valine) with phenylalanine, amino acid number 85 (isoleucine) with threonine, amino acid number 87 (phenylalanine) with tyrosine, amino acid number 99 (proline) with glutamine, amino acid number 103 (leucine) with valine, amino acid number 105 (leucine) with isoleucine, and amino acid number 108 (alanine) with threonine was designated as "hA2-11E-L2 type light chain".

[0376]

The amino acid sequence of the humanized hA2-11E-L2 type light chain is described in SEQ ID NO: 115 of the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20 of the amino acid sequence of SEQ ID NO: 115, a sequence consisting of amino acid numbers 21 to 128 thereof, and a sequence consisting of amino acid numbers 129 to 233 thereof correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 115 is described in SEQ ID NO: 114 of the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 60 of the nucleotide sequence of SEQ ID NO: 114, a sequence consisting of nucleotide numbers 61 to 384 thereof, and a sequence consisting of nucleotide numbers 385 to 699 thereof encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 114 and the amino acid sequence of SEQ ID NO: 115 are also described in Figure 40.

[0377]

17)-3-2 Humanized hA2-11E-L3 type light chain

A humanized hA2-11E light chain designed from the A2-11E light chain of SEQ ID NO: 4 of the Sequence Listing by the replacement of amino acid number 10 (leucine) with serine, amino acid number 21 (leucine) with isoleucine, amino acid number 22 (serine) with threonine, amino acid number 40 (leucine) with proline, amino acid number 42 (glutamic acid) with lysine, amino acid number 83 (valine) with phenylalanine, amino acid number 85 (isoleucine) with threonine, amino acid number 87 (phenylalanine) with tyrosine, amino acid number 99 (proline) with glutamine, amino acid number 103 (leucine) with valine, amino acid number 105 (leucine) with isoleucine, and amino acid number 108 (alanine) with threonine was designated as "hA2-11E-L3 type light chain".

[0378]

The amino acid sequence of the humanized hA2-11E-L3 type light chain is described in SEQ ID NO: 117 of the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20 of the amino acid sequence of SEQ ID NO: 117, a sequence consisting of amino acid numbers 21 to 128 thereof, and a sequence consisting of amino acid numbers 129 to 233 thereof correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 117 is described in SEQ ID NO: 116 of the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 60 of the nucleotide sequence of SEQ ID NO: 116, a sequence consisting of nucleotide numbers 61 to 384 thereof, and a sequence consisting of nucleotide numbers 385 to 699 thereof encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 116 and the amino acid sequence of SEQ ID NO: 117 are also described in Figure 41.

[0379]

17)-3-3 Humanized hA2-11E-L4 type light chain

A humanized hA2-11E light chain designed from the A2-11E light chain of SEQ ID NO: 4 of the Sequence Listing by the replacement of amino acid number 10 (leucine) with serine, amino acid number 21 (leucine) with isoleucine, amino acid number 40 (leucine) with proline, amino acid number 83 (valine) with phenylalanine, amino acid number 103 (leucine)

with valine, amino acid number 105 (leucine) with isoleucine, and amino acid number 108 (alanine) with threonine was designated as "hA2-11E-L4 type light chain".

[0380]

The amino acid sequence of the humanized hA2-11E-L4 type light chain is described in SEQ ID NO: 119 of the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20 of the amino acid sequence of SEQ ID NO: 119, a sequence consisting of amino acid numbers 21 to 128 thereof, and a sequence consisting of amino acid numbers 129 to 233 thereof correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 119 is described in SEQ ID NO: 118 of the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 60 of the nucleotide sequence of SEQ ID NO: 118, a sequence consisting of nucleotide numbers 61 to 384 thereof, and a sequence consisting of nucleotide numbers 385 to 699 thereof encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 118 and the amino acid sequence of SEQ ID NO: 119 are also described in Figure 42.

[0381]

17)-4 Design of humanized hA2-11E by combination of heavy chain and light chain

An antibody consisting of the humanized hA2-11E-H3 type heavy chain and the humanized hA2-11E-L2 type light chain was designed and designated as "humanized hA2-11E-H3/L2" (also referred to as "hA2-11E-H3/L2"). An antibody consisting of the humanized hA2-11E-H3 type heavy chain and the humanized hA2-11E-L3 type light chain was designed and designated as "humanized hA2-11E-H3/L3" (also referred to as "hA2-11E-H3/L3"). An antibody consisting of the humanized hA2-11E-H3 type heavy chain and the humanized hA2-11E-L4 type light chain was designed and designated as "humanized hA2-11E-H3/L4" (also referred to as "hA2-11E-H3/L4"). An antibody consisting of the humanized hA2-11E-H4 type heavy chain and the humanized hA2-11E-L2 type light chain was designed and designated as "humanized hA2-11E-H4/L2" (also referred to as "hA2-11E-H4/L2"). An antibody consisting of the humanized hA2-11E-H4 type heavy chain and the

humanized hA2-11E-L3 type light chain was designed and designated as "humanized hA2-11E-H4/L3" (also referred to as "hA2-11E-H4/L3"). An antibody consisting of the humanized hA2-11E-H4 type heavy chain and the humanized hA2-11E-L4 type light chain was designed and designated as "humanized hA2-11E-H4/L4" (also referred to as "hA2-11E-H4/L4"). These designed antibodies can be prepared according to Example 18 and evaluated according to Examples 2 and 4.

[0382]

17)-5 Design of humanized hA2-25C

17)-5-1 Molecular modeling of A2-25C variable regions

The molecular modeling of the A2-25C variable regions was carried out by a method generally known as homology modeling (Methods in Enzymology, 203, 121-153, (1991)). The variable regions of AA2-25C determined above were compared with 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)). As a result, 3BN9 was selected because of having the highest sequence identity to the heavy and light chain variable regions of A2-25C. The three-dimensional structures of framework regions were prepared as a "framework model" by combining the coordinates of 3BN9. Subsequently, the typical conformation of each CDR was incorporated into the framework model.

[0383]

Finally, energy minimization calculation for excluding disadvantageous interatomic contact was conducted in order to obtain possible molecular models of the A2-25C variable regions in terms of energy. These procedures were performed using a commercially available protein three-dimensional structure analysis program Discovery Studio (manufactured by Accelrys).

[0384]

17)-5-2 Design of amino acid sequence of humanized hA2-25C

Humanized hA2-25C was constructed by a method generally known as CDR grafting (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). An acceptor antibody was selected on the basis of the identity of amino acids in framework regions.

[0385]

The sequences of the framework regions of A2-25C were compared with the framework regions of human subgroup consensus sequences. As a result, a human γ chain subgroup 3 consensus sequence and a human κ chain subgroup 1 consensus sequence specified by KABAT et al. (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service National Institutes of Health, Bethesda, MD. (1991)) were selected as acceptors due to their high sequence identity as to framework regions. The amino acid residues of the framework regions of the human γ chain subgroup 3 consensus sequence and the human κ chain subgroup 1 consensus sequence were aligned with the amino acid residues of the A2-25C framework regions to identify the numbers of amino acids that did not match therebetween. The numbers of these residues were analyzed using the three-dimensional model of A2-25C constructed above. Then, the donor residues to be grafted onto the acceptors were selected according to the criteria provided by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). Some donor residues thus selected were transferred to the acceptor antibody to construct the humanized hA2-25C sequence as described in Examples below.

[0386]

17)-6 Humanization of A2-25C heavy chain

17)-6-1 Humanized hA2-25C-H3 type heavy chain

A humanized hA2-25C heavy chain designed from the A2-25C heavy chain of SEQ ID NO: 10 of the Sequence Listing by the replacement of amino acid number 19 (lysine) with arginine, amino acid number 38 (cysteine) with arginine, amino acid number 42 (threonine) with glycine, amino acid number 63 (threonine) with serine, amino acid number 75 (alanine) with serine, amino acid number 84 (aspartic acid) with asparagine, amino acid number 88 (serine) with alanine, amino acid number 93 (threonine) with valine, amino acid number 112

(valine) with threonine, and amino acid number 113 (methionine) with leucine was designated as "hA2-25C-H3 type heavy chain".

[0387]

The amino acid sequence of the humanized hA2-25C-H3 type heavy chain is described in SEQ ID NO: 121 of the Sequence Listing. A sequence consisting of amino acid numbers 1 to 19 of the amino acid sequence of SEQ ID NO: 121, a sequence consisting of amino acid numbers 20 to 137 thereof, and a sequence consisting of amino acid numbers 138 to 467 thereof correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 121 is described in SEQ ID NO: 120 of the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 57 of the nucleotide sequence of SEQ ID NO: 120, a sequence consisting of nucleotide numbers 58 to 411 thereof, and a sequence consisting of nucleotide numbers 412 to 1401 thereof encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 120 and the amino acid sequence of SEQ ID NO: 121 are also described in Figure 43.

[0388]

17)-6-2 Humanized hA2-25C-H4 type heavy chain

A humanized hA2-25C heavy chain designed from the A2-25C heavy chain of SEQ ID NO: 10 of the Sequence Listing by the replacement of amino acid number 19 (lysine) with arginine, amino acid number 38 (cysteine) with arginine, amino acid number 42 (threonine) with glycine, amino acid number 63 (threonine) with serine, amino acid number 75 (alanine) with serine, amino acid number 84 (aspartic acid) with asparagine, amino acid number 88 (serine) with alanine, and amino acid number 113 (methionine) with leucine was designated as "hA2-25C-H4 type heavy chain".

[0389]

The amino acid sequence of the humanized hA2-25C-H4 type heavy chain is described in SEQ ID NO: 123 of the Sequence Listing. A sequence consisting of amino acid numbers 1 to 19 of the amino acid sequence of SEQ ID NO: 123, a sequence consisting of amino acid

numbers 20 to 137 thereof, and a sequence consisting of amino acid numbers 138 to 467 thereof correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 123 is described in SEQ ID NO: 122 of the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 57 of the nucleotide sequence of SEQ ID NO: 122, a sequence consisting of nucleotide numbers 58 to 411 thereof, and a sequence consisting of nucleotide numbers 412 to 1401 thereof encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 122 and the amino acid sequence of SEQ ID NO: 123 are also described in Figure 44.

[0390]

17)-7 Humanization of A2-25C light chain

17)-7-1 Humanized hA2-25C-L1 type light chain

A humanized hA2-25C light chain designed from the A2-25C light chain of SEQ ID NO: 12 of the Sequence Listing by the replacement of amino acid number 9 (alanine) with serine, amino acid number 15 (leucine) with valine, amino acid number 16 (glutamic acid) with glycine, amino acid number 17 (glutamic acid) with aspartic acid, amino acid number 18 (isoleucine) with arginine, amino acid number 43 (serine) with alanine, amino acid number 45 (glutamine) with lysine, amino acid number 66 (arginine) with glycine, amino acid number 70 (glutamine) with aspartic acid, amino acid number 71 (tyrosine) with phenylalanine, amino acid number 72 (serine) with threonine, amino acid number 74 (lysine) with threonine, amino acid number 77 (arginine) with serine, amino acid number 79 (arginine) with glutamine, amino acid number 80 (valine) with proline, amino acid number 83 (isoleucine) with phenylalanine, amino acid number 84 (glycine) with alanine, amino acid number 85 (isoleucine) with threonine, amino acid number 100 (serine) with glutamine, amino acid number 104 (leucine) with valine, and amino acid number 109 (alanine) with threonine was designated as "hA2-25C-L1 type light chain".

[0391]

The amino acid sequence of the humanized hA2-25C-L1 type light chain is described in SEQ ID NO: 125 of the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20 of the amino acid sequence of SEQ ID NO: 125, a sequence consisting of amino acid numbers 21 to 129 thereof, and a sequence consisting of amino acid numbers 130 to 234 thereof correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 125 is described in SEQ ID NO: 124 of the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 60 of the nucleotide sequence of SEQ ID NO: 124, a sequence consisting of nucleotide numbers 61 to 387 thereof, and a sequence consisting of nucleotide numbers 388 to 702 thereof encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 124 and the amino acid sequence of SEQ ID NO: 125 are also described in Figure 45.

[0392]

17)-7-2 Humanized hA2-25C-L2 type light chain

A humanized hA2-25C light chain designed from the A2-25C light chain of SEQ ID NO: 12 of the Sequence Listing by the replacement of amino acid number 9 (alanine) with serine, amino acid number 15 (leucine) with valine, amino acid number 16 (glutamic acid) with glycine, amino acid number 17 (glutamic acid) with aspartic acid, amino acid number 18 (isoleucine) with arginine, amino acid number 43 (serine) with alanine, amino acid number 45 (glutamine) with lysine, amino acid number 70 (glutamine) with aspartic acid, amino acid number 72 (serine) with threonine, amino acid number 74 (lysine) with threonine, amino acid number 77 (arginine) with serine, amino acid number 79 (arginine) with glutamine, amino acid number 80 (valine) with proline, amino acid number 83 (isoleucine) with phenylalanine, amino acid number 84 (glycine) with alanine, amino acid number 85 (isoleucine) with threonine, amino acid number 100 (serine) with glutamine, amino acid number 104 (leucine) with valine, and amino acid number 109 (alanine) with threonine was designated as "hA2-25C-L2 type light chain".

[0393]

The amino acid sequence of the humanized hA2-25C-L2 type light chain is described in SEQ ID NO: 127 of the Sequence Listing. A sequence consisting of amino acid numbers 1 to 20 of the amino acid sequence of SEQ ID NO: 127, a sequence consisting of amino acid numbers 21 to 129 thereof, and a sequence consisting of amino acid numbers 130 to 234 thereof correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 127 is described in SEQ ID NO: 126 of the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 60 of the nucleotide sequence of SEQ ID NO: 126, a sequence consisting of nucleotide numbers 61 to 387 thereof, and a sequence consisting of nucleotide numbers 388 to 702 thereof encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 126 and the amino acid sequence of SEQ ID NO: 127 are also described in Figure 46.

[0394]

17)-7-3 Humanized hA2-25C-L3 type light chain

A humanized hA2-25C light chain designed from the A2-25C light chain of SEQ ID NO: 12 of the Sequence Listing by the replacement of amino acid number 9 (alanine) with serine, amino acid number 15 (leucine) with valine, amino acid number 16 (glutamic acid) with glycine, amino acid number 17 (glutamic acid) with aspartic acid, amino acid number 18 (isoleucine) with arginine, amino acid number 45 (glutamine) with lysine, amino acid number 72 (serine) with threonine, amino acid number 74 (lysine) with threonine, amino acid number 77 (arginine) with serine, amino acid number 79 (arginine) with glutamine, amino acid number 80 (valine) with proline, amino acid number 83 (isoleucine) with phenylalanine, amino acid number 84 (glycine) with alanine, amino acid number 85 (isoleucine) with threonine, amino acid number 104 (leucine) with valine, and amino acid number 109 (alanine) with threonine was designated as "hA2-25C-L3 type light chain".

[0395]

The amino acid sequence of the humanized hA2-25C-L3 type light chain is described in SEQ ID NO: 129 of the Sequence Listing. A sequence consisting of amino acid numbers

1 to 20 of the amino acid sequence of SEQ ID NO: 129, a sequence consisting of amino acid numbers 21 to 129 thereof, and a sequence consisting of amino acid numbers 130 to 234 thereof correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 129 is described in SEQ ID NO: 128 of the Sequence Listing. A sequence consisting of nucleotide numbers 1 to 60 of the nucleotide sequence of SEQ ID NO: 128, a sequence consisting of nucleotide numbers 61 to 387 thereof, and a sequence consisting of nucleotide numbers 388 to 702 thereof encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 128 and the amino acid sequence of SEQ ID NO: 129 are also described in Figure 47.

[0396]

17)-8 Design of humanized hA2-25C by combination of heavy chain and light chain

An antibody consisting of the humanized hA2-25C-H3 type heavy chain and the humanized hA2-25C-L1 type light chain was designed and designated as "humanized hA2-25C-H3/L1" (also referred to as "hA2-25C-H3/L1"). An antibody consisting of the humanized hA2-25C-H3 type heavy chain and the humanized hA2-25C-L2 type light chain was designed and designated as "humanized hA2-25C-H3/L2" (also referred to as "hA2-25C-H3/L2"). An antibody consisting of the humanized hA2-25C-H3 type heavy chain and the humanized hA2-25C-L3 type light chain was designed and designated as "humanized hA2-25C-H3/L3" (also referred to as "hA2-25C-H3/L3"). An antibody consisting of the humanized hA2-25C-H4 type heavy chain and the humanized hA2-25C-L1 type light chain was designed and designated as "humanized hA2-25C-H4/L1" (also referred to as "hA2-25C-H4/L1"). An antibody consisting of the humanized hA2-25C-H4 type heavy chain and the humanized hA2-25C-L2 type light chain was designed and designated as "humanized hA2-25C-H4/L2" (also referred to as "hA2-25C-H4/L2"). An antibody consisting of the humanized hA2-25C-H4 type heavy chain and the humanized hA2-25C-L3 type light chain was designed and designated as "humanized hA2-25C-H4/L3" (also referred to as "hA2-25C-

H4/L3"). These designed antibodies can be prepared according to Example 18 and evaluated according to Examples 2 and 4.

[0397]

<Example 18>

Construction and preparation of vectors for humanized A2-11E antibody (IgG1) and humanized A2-25C antibody (IgG1)

18)-1 Construction of humanized A2-11E heavy chain expression vector

18)-1-1 Construction of humanized hA2-11E-H3 type heavy chain expression vector

A DNA fragment containing a humanized hA2-11E-H3 variable region-encoding DNA sequence represented by nucleotide numbers 36 to 428 of the nucleotide sequence of humanized hA2-11E-H3 of SEQ ID NO: 110 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-11E-H3 expression vector was constructed in the same way as in Example 8)-1-1. The obtained expression vector was designated as "pCMA-G1/hA2-11E-H3".

[0398]

18)-1-2 Construction of humanized hA2-11E-H4 type heavy chain expression vector

A DNA fragment containing a humanized hA2-11E-H4 variable region-encoding DNA sequence represented by nucleotide numbers 36 to 428 of the nucleotide sequence of humanized hA2-11E-H4 of SEQ ID NO: 112 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-11E-H4 expression vector was constructed in the same way as in Example 8)-1-1. The obtained expression vector was designated as "pCMA-G1/hA2-11E-H4".

[0399]

18)-2 Construction of humanized A2-11E light chain expression vector

18)-2-1 Construction of humanized hA2-11E-L2 type light chain expression vector

A DNA fragment containing a humanized hA2-11E-L2 variable region-encoding DNA sequence represented by nucleotide numbers 37 to 399 of the nucleotide sequence of humanized hA2-11E-L2 of SEQ ID NO: 114 was synthesized (GeneArt Artificial Gene Synthesis Service). The DNA fragment containing a humanized hA2-11E-L2 variable

region-encoding DNA sequence was amplified using the synthesized DNA fragment as a template, KOD-Plus- (Toyobo Co., Ltd.), and a primer set given below, and inserted at the restriction enzyme BsiWI-cleaved site of the chimeric and humanized antibody light chain expression vector pCMA-LK constructed in Example 5)-1 using In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.) to construct a humanized hA2-11E-L2 expression vector. The obtained expression vector was designated as "pCMA/hA2-11E-L2".

Primer set

5'-CTGTGGATCTCCGGCGCGTACGGC-3' (CM-LKF; SEQ ID NO: 134)

5'-GGAGGGGGCGGCCACCGTACG-3' (KCL-Inf-R; SEQ ID NO: 135)

[0400]

18)-2-2 Construction of humanized hA2-11E-L3 type light chain expression vector

A DNA fragment containing a humanized hA2-11E-L3 variable region-encoding DNA sequence represented by nucleotide numbers 37 to 399 of the nucleotide sequence of humanized hA2-11E-L3 of SEQ ID NO: 116 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-11E-L3 expression vector was constructed in the same way as in Example 18)-2-1. The obtained expression vector was designated as "pCMA/hA2-11E-L3".

[0401]

18)-2-3 Construction of humanized hA2-11E-L4 type light chain expression vector

A DNA fragment containing a humanized hA2-11E-L4 variable region-encoding DNA sequence represented by nucleotide numbers 37 to 339 of the nucleotide sequence of humanized hA2-11E-L4 of SEQ ID NO: 118 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-11E-L4 expression vector was constructed in the same way as in Example 18)-2-1. The obtained expression vector was designated as "pCMA/hA2-11E-L4".

[0402]

18)-3 Construction of humanized A2-25C heavy chain expression vector

18)-3-1 Construction of humanized hA2-25C-H3 type heavy chain expression vector

A DNA fragment containing a humanized hA2-25C-H3 variable region-encoding DNA sequence represented by nucleotide numbers 36 to 428 of the nucleotide sequence of humanized hA2-25C-H3 of SEQ ID NO: 120 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-25C-H3 expression vector was constructed in the same way as in Example 8)-1-1. The obtained expression vector was designated as "pCMA-G1/hA2-25C-H3".

[0403]

18)-3-2 Construction of humanized hA2-25C-H4 type heavy chain expression vector

A DNA fragment containing a humanized hA2-25C-H4 variable region-encoding DNA sequence represented by nucleotide numbers 36 to 428 of the nucleotide sequence of humanized hA2-25C-H4 of SEQ ID NO: 122 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-25C-H4 expression vector was constructed in the same way as in Example 8)-1-1. The obtained expression vector was designated as "pCMA-G1/hA2-25C-H4".

[0404]

18)-4 Construction of humanized A2-25C light chain expression vector

18)-4-1 Construction of humanized hA2-25C-L1 type light chain expression vector

A DNA fragment containing a humanized hA2-25C-L1 variable region-encoding DNA sequence represented by nucleotide numbers 37 to 402 of the nucleotide sequence of humanized hA2-25C-L1 of SEQ ID NO: 124 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-25C-L1 expression vector was constructed in the same way as in Example 18)-2-1. The obtained expression vector was designated as "pCMA/hA2-25C-L1".

[0405]

18)-4-2 Construction of humanized hA2-25C-L2 type light chain expression vector

A DNA fragment containing a humanized hA2-25C-L2 variable region-encoding DNA sequence represented by nucleotide numbers 37 to 402 of the nucleotide sequence of humanized hA2-25C-L2 of SEQ ID NO: 126 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-25C-L2 expression vector was constructed in the

same way as in Example 18)-2-1. The obtained expression vector was designated as "pCMA/hA2-25C-L2".

[0406]

18)-4-3 Construction of humanized hA2-25C-L3 type light chain expression vector

A DNA fragment containing a humanized hA2-25C-L3 variable region-encoding DNA sequence represented by nucleotide numbers 37 to 402 of the nucleotide sequence of humanized hA2-25C-L3 of SEQ ID NO: 128 was synthesized (GeneArt Artificial Gene Synthesis Service). A humanized hA2-25C-L3 expression vector was constructed in the same way as in Example 18)-2-1. The obtained expression vector was designated as "pCMA/hA2-25C-L3".

[0407]

18)-5 Small-scale production of humanized A2-11E antibody (IgG1) and humanized A2-25C antibody (IgG1)

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

[0408]

1×10^7 FreeStyle 293F cells (Invitrogen Corp.) in the logarithmic growth phase were diluted to 9.6 mL with FreeStyle 293 expression medium (Invitrogen Corp.), then inoculated to 30 mL Square Storage Bottle (Nalgene/Thermo Fisher Scientific Inc.), and shake-cultured at 90 rpm at 37°C for 1 hour in an 8% CO₂ incubator. 30 µg of polyethyleneimine (Polysciences #24765) was dissolved in 200 µL of Opti-Pro SFM (Invitrogen Corp.). Next, each light chain expression vector (6 µg) and heavy chain expression vector (4 µg) prepared using NucleoBond Xtra (Takara Bio Inc.) were added to 200 µL of Opti-Pro SFM (Invitrogen Corp.). 200 µL of the expression vector/Opti-Pro SFM mixed solution was added to 200 µL of the polyethyleneimine/Opti-Pro SFM mixed solution, and the mixture was gently stirred, further left for 5 minutes, and then added to the FreeStyle 293F cells. The cells were shake-cultured at 90 rpm at 37°C for 7 days in an 8% CO₂ incubator, and the obtained culture supernatant was filtered through Minisart-Plus filter (Sartorius Japan K.K.) and used as a sample for evaluation.

[0409]

Humanized hA2-11E-H3/L2 was obtained by the combination of pCMA-G1/hA2-11E-H3 constructed in Example 18)-1-1 and pCMA/hA2-11E-L2 constructed in Example 18)-2-1. Humanized hA2-11E-H3/L3 was obtained by the combination of pCMA-G1/hA2-11E-H3 constructed in Example 18)-1-1 and pCMA/hA2-11E-L3 constructed in Example 18)-2-2. Humanized hA2-11E-H3/L4 was obtained by the combination of pCMA-G1/hA2-11E-H3 constructed in Example 18)-1-1 and pCMA/hA2-11E-L4 constructed in Example 18)-2-3. Humanized hA2-11E-H4/L2 was obtained by the combination of pCMA-G1/hA2-11E-H4 constructed in Example 18)-1-2 and pCMA/hA2-11E-L2 constructed in Example 18)-2-1. Humanized hA2-11E-H4/L3 was obtained by the combination of pCMA-G1/hA2-11E-H4 constructed in Example 18)-1-2 and pCMA/hA2-11E-L3 constructed in Example 18)-2-2. Humanized hA2-11E-H4/L4 was obtained by the combination of pCMA-G1/hA2-11E-H4 constructed in Example 18)-1-2 and pCMA/hA2-11E-L4 constructed in Example 18)-2-3.

[0410]

Humanized hA2-25C-H3/L1 was obtained by the combination of pCMA-G1/hA2-25C-H3 constructed in Example 18)-3-1 and pCMA-/hA2-25C-L1 constructed in Example 18)-4-1. Humanized hA2-25C-H3/L2 was obtained by the combination of pCMA-G1/hA2-25C-H3 constructed in Example 18)-3-1 and pCMA-/hA2-25C-L2 constructed in Example 18)-4-2. Humanized hA2-25C-H3/L3 was obtained by the combination of pCMA-G1/hA2-25C-H3 constructed in Example 18)-3-1 and pCMA-/hA2-25C-L3 constructed in Example 18)-4-3. Humanized hA2-25C-H4/L1 was obtained by the combination of pCMA-G1/hA2-25C-H4 constructed in Example 18)-3-2 and pCMA-/hA2-25C-L1 constructed in Example 18)-4-1. Humanized hA2-25C-H4/L2 was obtained by the combination of pCMA-G1/hA2-25C-H4 constructed in Example 18)-3-2 and pCMA-/hA2-25C-L2 constructed in Example 18)-4-2. Humanized hA2-25C-H4/L3 was obtained by the combination of pCMA-G1/hA2-25C-H4 constructed in Example 18)-3-2 and pCMA-/hA2-25C-L3 constructed in Example 18)-4-3.

[0411]

<Example 19>

Evaluation of *in vitro* activity of humanized A2-11E antibody (IgG1) and humanized A2-25C antibody (IgG1)

19)-1 Evaluation of the antibodies in BMP-induced osteoblast differentiation assay

The humanized A2-11E antibodies (IgG1) and the humanized A2-25C antibodies (IgG1) prepared in Example 18)-5 were analyzed for their inhibitory activity against intracellular signals through endogenous ALK2, on the basis of their effects on BMP-induced osteoblast differentiation assay using C2C12 cells in the same way as in Example 6)-2. 2.5 ng/mL GDF2/BMP9 (manufactured by R&D Systems, Inc.) was used in the differentiation induction.

[0412]

The results are shown in Figure 48. The humanized A2-11E antibodies (IgG1) and the humanized A2-25C antibodies (IgG1) were confirmed to inhibit, in a dose-dependent manner, the differentiation of C2C12 cells into osteoblast-like cells induced by BMP.

[0413]

<Example 20>

Evaluation of binding activity of humanized A2-11E antibody (IgG1) and humanized A2-25C antibody (IgG1) against human ALK2

The dissociation constants of the humanized A2-11E antibodies (IgG1) and the humanized A2-25C antibodies (IgG1) prepared in Example 18)-5 for the antigen (human ALK2 extracellular domain prepared in Example 14)-1) were measured using Biacore T200 (GE Healthcare Bio-Sciences Corp.) by the capture method which involves capturing each antibody as a ligand onto an immobilized anti-human IgG (Fc) antibody and assaying the antigen as an analyte. Approximately 1000 RU of the anti-human IgG (Fc) antibody (Human Antibody Capture kit, GE Healthcare Bio-Sciences Corp.) was covalently bound to a sensor chip CM5 (GE Healthcare Bio-Sciences Corp.) by the amine coupling method. Similarly, this antibody was immobilized onto a reference cell. The running buffer used was HBS-EP+ (10 mM HEPES (pH 7.4), 0.15 M NaCl, 3 mM EDTA, and 0.05% Surfactant P20). The culture supernatant containing the antibody was added onto the anti-human IgG (Fc) antibody-immobilized chip for approximately 1 minute. Then, serially diluted solutions (0.78 to 200

nM) of the antigen were added thereto at a flow rate of 30 µl/min for 300 seconds. Subsequently, the dissociation phase was monitored for 600 seconds. 3 M MgCl₂ was added thereto as a regenerating solution at a flow rate of 10 µl/min for 30 seconds. The data was analyzed using 1:1 binding models of analytical software (BIAevaluation software, version 1.0) to calculate an association rate constant *k_a*, a dissociation rate constant *k_d*, and a dissociation constant (KD; KD = *k_d*/*k_a*).

[0414]

The results are shown in Table 3.

[Table 3]

Dissociation constants of humanized A2-11E antibody and humanized A2-25C antibody

	Name	KD(nM)
1	hA2-11E-H3/L2	63.1
2	hA2-11E-H3/L3	47.4
3	hA2-11E-H3/L4	31.3
4	hA2-11E-H4/L2	19.9
5	hA2-11E-H4/L3	18.5
6	hA2-11E-H4/L4	11.1
7	hA2-25C-H3/L1	115.8
8	hA2-25C-H3/L2	53.3
9	hA2-25C-H3/L3	64.4
10	hA2-25C-H4/L1	81.8
11	hA2-25C-H4/L2	56.7
12	hA2-25C-H4/L3	27.9

[0415]

<Example 21>

Epitope analysis of A2-25C antibody

21)-1 Preparation of human chimeric cA2-25C Fab fragment

Human chimeric cA2-25C prepared in the same way as in Example 5)-8 was cleaved into Fab and Fc fragments using papain (Sigma-Aldrich Co. LLC), and these fragments were added to HiTrap Protein A HP column (GE Healthcare Bio-Sciences Corp.). The Fab fragment recovered as a flow-through fraction was concentrated.

[0416]

21)-2 Crystallization and structural analysis of human chimeric cA2-25C Fab fragment and ALK2-ECD complex

The protein complex of the chimeric A2-25C Fab fragment obtained in Example 21)-1 and the ALK2-ECD prepared according to Example 14 were concentrated to 3.8 mg/mL and used in crystallization trial employing vapor diffusion method. To 0.5 μ L of the protein solution, an equal amount of a precipitant solution (0.15 M Li_2SO_4 , 0.1 M Na citrate (pH 3.4), 18% (w/v) PEG6,000, and 20% (v/v) ethylene glycol) was added, and the resulting solution was placed in a sealed container containing 50 μ L of a precipitant solution such that these solutions had no contact with each other. The container was left standing at 20°C. Three days later, 0.1 mm $\times$ 0.05 mm $\times$ 0.02 mm single crystals were obtained. The obtained crystals were frozen in liquid nitrogen. X-ray diffraction data was collected under 95 K nitrogen stream using BL41XU of Spring8. Diffraction intensity was digitized from the obtained diffraction image using software mosflm (CCP4: Collaborative Computational Project No. 4) to determine crystal structure factors. The crystals were in the orthorhombic crystal system with a space group $\text{P2}_1\text{2}_1\text{2}_1$ and unit cells of $a = 74.49$ angstroms, $b = 128.05$ angstroms, and $c = 147.73$ angstroms. The molecular replacement method was performed using the obtained structure factors and the three-dimensional structure coordinates of Fab (antibody structure determined by the past crystal structure analysis was used) to determine the phases. Software phaser (CCP4: Collaborative Computational Project No. 4) was used in calculation. The crystal contained two complexes in the asymmetric unit. Structure refinement was performed using software Refmac5 (CCP4), and model correction was performed using software coot. This operation was repetitively performed to obtain a final R factor of 22.4% and a free R factor of 25.3% with a resolution of 2.1 angstroms. The model consists of two complexes and contains amino acid residues 1 to 212 of the A2-25C Fab L chain, amino acid residues 1 to 219 of the A2-25C Fab H chain, amino acid residues 12 to 52 and 66 to 88 of ALK2-ECD, and 411 water molecules. The determined amino acid residues of ALK2-ECD commonly located within 4 angstroms from two A2-25C Fabs are as follows:

Glu18, Gly19, Leu20, Ile39, Asp41, Gly42, Phe43, His44, Val45, Tyr46, and Thr84. The ribbon model of the whole complex is shown in Figure 49.

[0417]

<Example 22>

Evaluation of inhibitory activity of A2-27D against various ALK2 mutants

The inhibitory activity of A2-27 against 13 types of mutants (L196P, delP197_F198insL, R202I, R206H, Q207E, R258S, R258G, G325A, G328E, G328R, G328W, G356D, and R375P) identified so far from FOP cases, and wild-type ALK2 was analyzed in the same way as in Example 2)-3 using HEK293A cells and BMP-specific Id1WT4F-luc luciferase reporter. 2.5 hours after transfection, the medium was replaced with fresh OPTI-MEM I (manufactured by Life Technologies Corp.) containing 10 ng/mL BMP7 (manufactured by Miltenyi Biotec) and 3 µg/mL rat IgG1 (manufactured by R&D Systems, Inc.) or A2-27D, and the cells were cultured overnight. On the next day, luciferase activity was measured using Dual-Glo Luciferase Assay System (manufactured by Promega Corp.).

[0418]

The results are shown in Figure 50. The monoclonal antibody A2-27D was confirmed to inhibit luciferase activity induced by BMP7 in the wild-type ALK2 and all of the 13 types of mutants (Figure 50).

INDUSTRIAL APPLICABILITY

[0419]

The chimeric or humanized anti-ALK2 antibody of the present invention has an inhibitory effect on ALK2-mediated BMP signal transduction. The pharmaceutical composition comprising the anti-ALK2 antibody can serve as a therapeutic or prophylactic drug for ectopic ossification and/or bone dysplasia, anemia, or diffuse intrinsic pontine glioma (DIPG).

FREE TEXT OF SEQUENCE LISTING

[0420]

SEQ ID NO: 1: Nucleotide sequence of cDNA encoding the heavy chain variable region of A2-11E

SEQ ID NO: 2: Amino acid sequence of the heavy chain variable region of A2-11E

SEQ ID NO: 3: Nucleotide sequence of cDNA encoding the light chain variable region of A2-11E

SEQ ID NO: 4: Amino acid sequence of the light chain variable region of A2-11E

SEQ ID NO: 5: Nucleotide sequence of cDNA encoding the heavy chain variable region of A2-15A

SEQ ID NO: 6: Amino acid sequence of the heavy chain variable region of A2-15A

SEQ ID NO: 7: Nucleotide sequence of cDNA encoding the light chain variable region of A2-15A

SEQ ID NO: 8: Amino acid sequence of the light chain variable region of A2-15A

SEQ ID NO: 9: Nucleotide sequence of cDNA encoding the heavy chain variable region of A2-25C

SEQ ID NO: 10: Amino acid sequence of the heavy chain variable region of A2-25C

SEQ ID NO: 11: Nucleotide sequence of cDNA encoding the light chain variable region of A2-25C

SEQ ID NO: 12: Amino acid sequence of the light chain variable region of A2-25C

SEQ ID NO: 13: Nucleotide sequence of cDNA encoding the heavy chain variable region of A2-27D

SEQ ID NO: 14: Amino acid sequence of the heavy chain variable region of A2-27D

SEQ ID NO: 15: Nucleotide sequence of cDNA encoding the light chain variable region of A2-27D

SEQ ID NO: 16: Amino acid sequence of the light chain variable region of A2-27D

SEQ ID NO: 17: Nucleotide sequence of a DNA fragment containing a sequence encoding the amino acids of a human κ chain secretory signal and a human κ chain constant region

SEQ ID NO: 18: Nucleotide sequence of a DNA fragment containing a sequence encoding the amino acids of a human heavy chain secretory signal and a human IgG1 constant region

SEQ ID NO: 19: Nucleotide sequence of the heavy chain of human chimeric cA2-15A

SEQ ID NO: 20: Amino acid sequence of the heavy chain of human chimeric cA2-15A
SEQ ID NO: 21: Nucleotide sequence of the light chain of human chimeric cA2-15A
SEQ ID NO: 22: Amino acid sequence of the light chain of human chimeric cA2-15A
SEQ ID NO: 23: Nucleotide sequence of the heavy chain of human chimeric cA2-27D
SEQ ID NO: 24: Amino acid sequence of the heavy chain of human chimeric cA2-27D
SEQ ID NO: 25: Nucleotide sequence of the light chain of human chimeric cA2-27D
SEQ ID NO: 26: Amino acid sequence of the light chain of human chimeric cA2-27D
SEQ ID NO: 27: Nucleotide sequence of humanized hA2-15A-H1
SEQ ID NO: 28: Amino acid sequence of humanized hA2-15A-H1
SEQ ID NO: 29: Nucleotide sequence of humanized hA2-15A-H4
SEQ ID NO: 30: Amino acid sequence of humanized hA2-15A-H4
SEQ ID NO: 31: Nucleotide sequence of a DNA fragment containing a sequence encoding humanized hA2-15A-L1
SEQ ID NO: 32: Amino acid sequence of humanized hA2-15A-L1
SEQ ID NO: 33: Nucleotide sequence of a DNA fragment containing a sequence encoding humanized hA2-15A-L4
SEQ ID NO: 34: Amino acid sequence of humanized hA2-15A-L4
SEQ ID NO: 35: Nucleotide sequence of a DNA fragment containing a sequence encoding humanized hA2-15A-L6
SEQ ID NO: 36: Amino acid sequence of humanized hA2-15A-L6
SEQ ID NO: 37: Nucleotide sequence of a DNA fragment containing a sequence encoding humanized hA2-15A-L7
SEQ ID NO: 38: Amino acid sequence of humanized hA2-15A-L7
SEQ ID NO: 39: Nucleotide sequence of humanized hA2-27D-H1
SEQ ID NO: 40: Amino acid sequence of humanized hA2-27D-H1
SEQ ID NO: 41: Nucleotide sequence of humanized hA2-27D-H2
SEQ ID NO: 42: Amino acid sequence of humanized hA2-27D-H2
SEQ ID NO: 43: Nucleotide sequence of humanized hA2-27D-H3
SEQ ID NO: 44: Amino acid sequence of humanized hA2-27D-H3

SEQ ID NO: 45: Nucleotide sequence of humanized hA2-27D-H4

SEQ ID NO: 46: Amino acid sequence of humanized hA2-27D-H4

SEQ ID NO: 47: Nucleotide sequence of humanized hA2-27D-H5

SEQ ID NO: 48: Amino acid sequence of humanized hA2-27D-H5

SEQ ID NO: 49: Nucleotide sequence of a DNA fragment containing a sequence encoding humanized hA2-27D-L1

SEQ ID NO: 50: Amino acid sequence of humanized hA2-27D-L1

SEQ ID NO: 51: Nucleotide sequence of a DNA fragment containing a sequence encoding humanized hA2-27D-L2

SEQ ID NO: 52: Amino acid sequence of humanized hA2-27D-L2

SEQ ID NO: 53: Nucleotide sequence of a DNA fragment containing a sequence encoding humanized hA2-27D-L3

SEQ ID NO: 54: Amino acid sequence of humanized hA2-27D-L3

SEQ ID NO: 55: Nucleotide sequence of a DNA fragment containing a sequence encoding humanized hA2-27D-L4

SEQ ID NO: 56: Amino acid sequence of humanized hA2-27D-L4

SEQ ID NO: 57: Nucleotide sequence of a DNA fragment containing a sequence encoding humanized hA2-27D-L5

SEQ ID NO: 58: Amino acid sequence of humanized hA2-27D-L5

SEQ ID NO: 59: Amino acid sequence of A2-15A CDRH1

SEQ ID NO: 60: Amino acid sequence of A2-15A CDRH2

SEQ ID NO: 61: Amino acid sequence of A2-15A CDRH3

SEQ ID NO: 62: Amino acid sequence of A2-15A CDRL1

SEQ ID NO: 63: Amino acid sequence of A2-15A CDRL2

SEQ ID NO: 64: Amino acid sequence of A2-15A CDRL3

SEQ ID NO: 65: Amino acid sequence of A2-27D CDRH1

SEQ ID NO: 66: Amino acid sequence of A2-27D CDRH2

SEQ ID NO: 67: Amino acid sequence of A2-27D CDRH3

SEQ ID NO: 68: Amino acid sequence of A2-27D CDRL1

SEQ ID NO: 69: Amino acid sequence of A2-27D CDRL2

SEQ ID NO: 70: Amino acid sequence of A2-27D CDRL3

SEQ ID NO: 71: Amino acid sequence of humanized hA2-15A-L6 CDRL2

SEQ ID NO: 72: Amino acid sequence of A2-11E CDRH1

SEQ ID NO: 73: Amino acid sequence of A2-11E CDRH2

SEQ ID NO: 74: Amino acid sequence of A2-11E CDRH3

SEQ ID NO: 75: Amino acid sequence of A2-11E CDRL1

SEQ ID NO: 76: Amino acid sequence of A2-11E CDRL2

SEQ ID NO: 77: Amino acid sequence of A2-11E CDRL3

SEQ ID NO: 78: Amino acid sequence of A2-25C CDRH1

SEQ ID NO: 79: Amino acid sequence of A2-25C CDRH2

SEQ ID NO: 80: Amino acid sequence of A2-25C CDRH3

SEQ ID NO: 81: Amino acid sequence of A2-25C CDRL1

SEQ ID NO: 82: Amino acid sequence of A2-25C CDRL2

SEQ ID NO: 83: Amino acid sequence of A2-25C CDRL3

SEQ ID NO: 84: Amino acid sequence of human ALK2

SEQ ID NO: 85: Nucleotide sequence of human ALK2

SEQ ID NO: 86: Amino acid sequence of mouse ALK2

SEQ ID NO: 87: Nucleotide sequence of mouse ALK2

SEQ ID NOs: 88 to 102: Primer

SEQ ID NO: 103: Nucleotide sequence of a DNA fragment containing a sequence encoding the amino acids of a human heavy chain signal sequence and a human IgG2 constant region

SEQ ID NO: 104: Nucleotide sequence of humanized hA2-15A-H4 IgG2 type

SEQ ID NO: 105: Amino acid sequence of humanized hA2-15A-H4 IgG2 type

SEQ ID NO: 106: Nucleotide sequence of humanized hA2-27D-H2-LALA

SEQ ID NO: 107: Amino sequence of humanized hA2-27D-H2-LALA

SEQ ID NO: 108: Nucleotide sequence of humanized hA2-27D-H3-LALA

SEQ ID NO: 109: Amino sequence of humanized hA2-27D-H3-LALA

SEQ ID NO: 110: Nucleotide sequence of humanized hA2-11E-H3

SEQ ID NO: 111: Amino acid sequence of humanized hA2-11E-H3
SEQ ID NO: 112: Nucleotide sequence of humanized hA2-11E-H4
SEQ ID NO: 113: Amino acid sequence of humanized hA2-11E-H4
SEQ ID NO: 114: Nucleotide sequence of humanized hA2-11E-L2
SEQ ID NO: 115: Amino acid sequence of humanized hA2-11E-L2
SEQ ID NO: 116: Nucleotide sequence of humanized hA2-11E-L3
SEQ ID NO: 117: Amino acid sequence of humanized hA2-11E-L3
SEQ ID NO: 118: Nucleotide sequence of humanized hA2-11E-L4
SEQ ID NO: 119: Amino acid sequence of humanized hA2-11E-L4
SEQ ID NO: 120: Nucleotide sequence of humanized hA2-25C-H3
SEQ ID NO: 121: Amino acid sequence of humanized hA2-25C-H3
SEQ ID NO: 122: Nucleotide sequence of humanized hA2-25C-H4
SEQ ID NO: 123: Amino acid sequence of humanized hA2-25C-H4
SEQ ID NO: 124: Nucleotide sequence of humanized hA2-25C-L1
SEQ ID NO: 125: Amino acid sequence of humanized hA2-25C-L1
SEQ ID NO: 126: Nucleotide sequence of humanized hA2-25C-L2
SEQ ID NO: 127: Amino acid sequence of humanized hA2-25C-L2
SEQ ID NO: 128: Nucleotide sequence of humanized hA2-25C-L3
SEQ ID NO: 129: Amino acid sequence of humanized hA2-25C-L3
SEQ ID NOs: 130 to 135: Primer

[0421]

All publications, patents, and patent applications cited herein are incorporated herein by reference in their entirety.

CLAIMS

1. An antibody or an antigen-binding fragment thereof which specifically binds to a polypeptide sequence consisting of at least 7 amino acids in any one of the following amino acid sequences (a) to (e) and inhibits ALK2-mediated BMP signal transduction:

- (a) the amino acid sequence of SEQ ID NO: 84;
- (b) an amino acid sequence consisting of amino acid numbers 21 to 123 of the amino acid sequence of SEQ ID NO: 84;
- (c) the amino acid sequence of SEQ ID NO: 86;
- (d) an amino acid sequence consisting of amino acid numbers 21 to 123 of the amino acid sequence of SEQ ID NO: 86; and
- (e) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in any one of the amino acid sequences (a) to (d).

2. An antibody or an antigen-binding fragment thereof which specifically binds to a polypeptide sequence consisting of at least 7 amino acids in an amino acid sequence encoded by any one of the following nucleotide sequences (f) to (j) and inhibits ALK2-mediated BMP signal transduction:

- (f) the nucleotide sequence of SEQ ID NO: 85;
- (g) a nucleotide sequence consisting of nucleotide numbers 728 to 1036 of the nucleotide sequence of SEQ ID NO: 85;
- (h) the nucleotide sequence of SEQ ID NO: 87;
- (i) a nucleotide sequence consisting of nucleotide numbers 728 to 1036 of the nucleotide sequence of SEQ ID NO: 87; and
- (j) a nucleotide sequence of a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one of the nucleotide sequences (f) to (i).

3. The antibody or the antigen-binding fragment thereof according to claim 1 or 2, wherein the polypeptide sequence is a sequence in an ALK2 extracellular region.

4. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 3, wherein the antibody binds to a wild-type ALK2 protein and a mutant ALK2 protein.

5. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 4, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

6. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 5, wherein the antibody or the antigen-binding fragment is a chimeric antibody, a humanized antibody, a human antibody, single-chain Fv, a bispecific antibody, or a multispecific antibody.

7. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 6, wherein the antibody cross-competes, for binding to the polypeptide, with at least any one antibody selected from the group consisting of an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 2 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 4, an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 6 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 8, an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 10 and a light chain variable region consisting of an amino acid sequence of SEQ ID NO: 12, and an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 14 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 16.

8. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 6, wherein the antibody binds to an epitope that is bound by at least any one antibody selected from the group consisting of an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 2 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 4, an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 6 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 8, an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 10 and a light chain variable region consisting of the amino acid sequence of SEQ ID

NO: 12, and an antibody comprising a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 14 and a light chain variable region consisting of the amino acid sequence of SEQ ID NO: 16.

9. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 6, wherein the antibody binds to an epitope comprising each residue of glutamic acid (Glu) at position 18, glycine (Gly) at position 19, isoleucine (Ile) at position 39, asparagine (Asn) at position 40, aspartic acid (Asp) at position 41, glycine (Gly) at position 42, phenylalanine (Phe) at position 43, histidine (His) at position 44, valine (Val) at position 45, tyrosine (Tyr) at position 46, asparagine (Asn) at position 82, threonine (Thr) at position 84, glutamine (Gln) at position 86, leucine (Leu) at position 87, proline (Pro) at position 88, and threonine (Thr) at position 89 in the amino acid sequence of SEQ ID NO: 84.

10. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 6, wherein the antibody has an interaction distance between the antibody or the antigen-binding fragment and each residue of glutamic acid (Glu) at position 18, glycine (Gly) at position 19, isoleucine (Ile) at position 39, asparagine (Asn) at position 40, aspartic acid (Asp) at position 41, glycine (Gly) at position 42, phenylalanine (Phe) at position 43, histidine (His) at position 44, valine (Val) at position 45, tyrosine (Tyr) at position 46, asparagine (Asn) at position 82, threonine (Thr) at position 84, glutamine (Gln) at position 86, leucine (Leu) at position 87, proline (Pro) at position 88, and threonine (Thr) at position 89 in the amino acid sequence of SEQ ID NO: 84.

11. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 6, wherein the antibody binds to an epitope comprising each residue of glutamic acid (Glu) at position 18, glycine (Gly) at position 19, leucine (Leu) at position 20, isoleucine (Ile) at position 39, asparagine (Asn) at position 40, aspartic acid (Asp) at position 41, glycine (Gly) at position 42, phenylalanine (Phe) at position 43, histidine (His) at position 44, valine (Val) at position 45, tyrosine (Tyr) at position 46, and threonine (Thr) at position 84 in the amino acid sequence of SEQ ID NO: 84.

12. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 6, wherein the antibody has an interaction distance between the antibody or the

antigen-binding fragment and each residue of glutamic acid (Glu) at position 18, glycine (Gly) at position 19, leucine (Leu) at position 20, isoleucine (Ile) at position 39, asparagine (Asn) at position 40, aspartic acid (Asp) at position 41, glycine (Gly) at position 42, phenylalanine (Phe) at position 43, histidine (His) at position 44, valine (Val) at position 45, tyrosine (Tyr) at position 46, and threonine (Thr) at position 84 in the amino acid sequence of SEQ ID NO: 84.

13. The antibody or the antigen-binding fragment thereof according to claim 10 or 12, wherein the interaction distance is 6 angstroms or smaller.

14. The antibody or the antigen-binding fragment thereof according to claim 10 or 12, wherein the interaction distance is 4 angstroms or smaller.

15. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 8, wherein

a heavy chain sequence comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1 consists of the amino acid sequence of SEQ ID NO: 72, the CDRH2 consists of the amino acid sequence of SEQ ID NO: 73, and the CDRH3 consists of the amino acid sequence of SEQ ID NO: 74; and

a light chain sequence comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1 consists of the amino acid sequence of SEQ ID NO: 75, the CDRL2 consists of the amino acid sequence of SEQ ID NO: 76, and the CDRL3 consists of the amino acid sequence of SEQ ID NO: 77.

16. The antibody or the antigen-binding fragment thereof according to claim 15, wherein the antibody comprises a heavy chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 2 and a light chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 4.

17. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 8, wherein

a heavy chain sequence comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1 consists of the amino acid sequence of SEQ ID NO: 59, the CDRH2 consists of the amino acid sequence of SEQ ID NO: 60, and the CDRH3 consists of the amino acid sequence of SEQ ID NO: 61; and

a light chain sequence comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1 consists of the amino acid sequence of SEQ ID NO: 62, the CDRL2 consists of the amino acid sequence of SEQ ID NO: 63 or SEQ ID NO: 71, and the CDRL3 consists of the amino acid sequence of SEQ ID NO: 64.

18. The antibody or the antigen-binding fragment thereof according to claim 17, wherein the antibody comprises a heavy chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 6 and a light chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 8 or amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 36.

19. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 10, 13, and 14, wherein

a heavy chain sequence comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1 consists of the amino acid sequence of SEQ ID NO: 78, the CDRH2 consists of the amino acid sequence of SEQ ID NO: 79, and the CDRH3 consists of the amino acid sequence of SEQ ID NO: 80; and

a light chain sequence comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1 consists of the amino acid sequence of SEQ ID NO: 81, the CDRL2 consists of the amino acid sequence of SEQ ID NO: 82, and the CDRL3 consists of the amino acid sequence of SEQ ID NO: 83.

20. The antibody or the antigen-binding fragment thereof according to claim 19, wherein the antibody comprises a heavy chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 10 and a light chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 12.

21. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 8 and 11 to 14, wherein

a heavy chain sequence comprises a variable region having CDRH1, CDRH2, and CDRH3, wherein the CDRH1 consists of the amino acid sequence of SEQ ID NO: 65, the CDRH2 consists of the amino acid sequence of SEQ ID NO: 66, and the CDRH3 consists of the amino acid sequence of SEQ ID NO: 67; and

a light chain sequence comprises a variable region having CDRL1, CDRL2, and CDRL3, wherein the CDRL1 consists of the amino acid sequence of SEQ ID NO: 68, the CDRL2 consists of the amino acid sequence of SEQ ID NO: 69, and the CDRL3 consists of the amino acid sequence of SEQ ID NO: 70.

22. The antibody or the antigen-binding fragment thereof according to claim 21, wherein the antibody comprises a heavy chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 14 and a light chain variable region sequence consisting of the amino acid sequence of SEQ ID NO: 16.

23. The antigen-binding fragment thereof according to any one of claims 1 to 22, wherein the antigen-binding fragment is selected from the group consisting of Fab, F(ab')₂, Fab', and Fv.

24. The antibody according to any one of claims 1 to 22, wherein the antibody is scFv.

25. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 22, wherein the antibody is a chimeric antibody.

26. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 22, wherein the antibody is humanized.

27. The antibody according to any one of claims 1 to 24, wherein the heavy chain comprises a constant region of a human immunoglobulin G1 heavy chain, a human immunoglobulin G2 heavy chain, or a human immunoglobulin G4 heavy chain, and the light chain comprises a constant region of a human immunoglobulin κ light chain.

28. The antibody according to claim 27, wherein the heavy chain comprises a constant region of a human immunoglobulin G1 heavy chain.

29. The antibody according to claim 28, wherein in the human immunoglobulin G1 heavy chain, leucine (Leu) at position 234 is substituted by alanine (Ala), and leucine (Leu) at position 235 is substituted by alanine (Ala).

30. The antibody according to claim 27, wherein the heavy chain comprises a constant region of a human immunoglobulin G2 heavy chain.

31. The antibody according to claim 27, wherein the heavy chain comprises a constant region of a human immunoglobulin G4 heavy chain.

32. The antibody according to claim 31, wherein in the human immunoglobulin G4 heavy chain, serine (Ser) at position 241 is substituted by proline (Pro).

33. An antibody or an antigen-binding fragment thereof which specifically binds to an extracellular region of ALK2 protein and inhibits ALK2-mediated BMP signal transduction, wherein the antibody comprises

(a) a heavy chain variable region sequence selected from the group consisting of the following amino acid sequences:

a1) an amino acid sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 28;

a2) an amino acid sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 30;

a3) an amino acid sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 105;

a4) an amino acid sequence having at least 95% identity to any of the amino acid sequences a1) to a3);

a5) an amino acid sequence having at least 99% identity to any of the amino acid sequences a1) to a3); and

a6) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in the amino acid sequences a1) to a3); and

(b) a light chain variable region sequence selected from the group consisting of the following amino acid sequences:

b1) an amino acid sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 32;

b2) an amino acid sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 34;

b3) an amino acid sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 36;

b4) an amino acid sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 38;

b5) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences b1) to b4);

b6) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences b1) to b4); and

b7) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in any one amino acid sequence selected from the amino acid sequences b1) to b4).

34. The antibody or the antigen-binding fragment thereof according to claim 33, wherein the antibody is an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 30 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 36, or an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 142 of the amino acid sequence of SEQ ID NO: 105 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 133 of the amino acid sequence of SEQ ID NO: 36.

35. The antibody according to claim 33, wherein the antibody is an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 472 of the amino acid sequence of SEQ ID NO: 30 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 36, or an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 468 of the amino acid sequence of SEQ ID NO: 105 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 36.

36. The antibody according to claim 33, wherein the antibody is an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 468 of the amino acid sequence of SEQ ID NO: 105 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 238 of the amino acid sequence of SEQ ID NO: 36.

37. An antibody or an antigen-binding fragment thereof which specifically binds to an extracellular region of ALK2 protein and inhibits ALK2-mediated BMP signal transduction, wherein the antibody comprises

(a) a heavy chain variable region sequence selected from the group consisting of the following amino acid sequences:

a1) an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 40;

a2) an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 42;

a3) an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 44;

a4) an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 46;

a5) an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 48;

a6) an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 107;

a7) an amino acid sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 109;

a8) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences a1) to a7);

a9) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences a1) to a7); and

a10) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in any one amino acid sequence selected from the amino acid sequences a1) to a7); and

(b) a light chain variable region sequence selected from the group consisting of the following amino acid sequences:

- b1) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 50;
- b2) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 52;
- b3) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 54;
- b4) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 56;
- b5) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 58;
- b6) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences b1) to b5);
- b7) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences b1) to b5); and
- b8) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in any one amino acid sequence selected from the amino acid sequences b1) to b5).

38. The antibody or the antigen-binding fragment thereof according to claim 37, wherein the antibody is an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 42 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 52, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 44 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 56, an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 107 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21

to 129 of the amino acid sequence of SEQ ID NO: 52, or an antibody consisting of a heavy chain comprising a heavy chain variable region sequence consisting of amino acid numbers 20 to 140 of the amino acid sequence of SEQ ID NO: 109 and a light chain comprising a light chain variable region sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 56.

39. The antibody according to claim 37, wherein the antibody is an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 42 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 52, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 44 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 56, an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 107 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 52, or an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 109 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 56.

40. The antibody according to claim 37, wherein the antibody is an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 107 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 52, or an antibody consisting of a heavy chain comprising an amino acid sequence consisting of amino acid numbers 20 to 470 of the amino acid sequence of SEQ ID NO: 109 and a light chain comprising an amino acid sequence consisting of amino acid numbers 21 to 234 of the amino acid sequence of SEQ ID NO: 56.

41. An antibody or an antigen-binding fragment thereof which specifically binds to an extracellular region of ALK2 protein and inhibits ALK2-mediated BMP signal transduction, wherein the antibody comprises

(a) a heavy chain variable region sequence selected from the group consisting of the following amino acid sequences:

a1) an amino acid sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 111;

a2) an amino acid sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 113;

a3) an amino acid sequence having at least 95% identity to the amino acid sequence a1) or a2);

a4) an amino acid sequence having at least 99% identity to the amino acid sequence a1) or a2);

and

a5) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in the amino acid sequence a1) or a2); and

(b) a light chain variable region sequence selected from the group consisting of the following amino acid sequences:

b1) an amino acid sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 115;

b2) an amino acid sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 117;

b3) an amino acid sequence consisting of amino acid numbers 21 to 128 of the amino acid sequence of SEQ ID NO: 119;

b4) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences b1) to b3);

b5) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences b1) to b3); and

b6) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in any one amino acid sequence selected from the amino acid sequences b1) to b3).

42. An antibody or an antigen-binding fragment thereof which specifically binds to an extracellular region of ALK2 protein and inhibits ALK2-mediated BMP signal transduction, wherein the antibody comprises

(a) a heavy chain variable region sequence selected from the group consisting of the following amino acid sequences:

a1) an amino acid sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 121;

a2) an amino acid sequence consisting of amino acid numbers 20 to 137 of the amino acid sequence of SEQ ID NO: 123;

a3) an amino acid sequence having at least 95% identity to the amino acid sequence a1) or a2);

a4) an amino acid sequence having at least 99% identity to the amino acid sequence a1) or a2);

and

a5) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in the amino acid sequence a1) or a2); and

(b) a light chain variable region sequence selected from the group consisting of the following amino acid sequences:

b1) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 125;

b2) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 127;

b3) an amino acid sequence consisting of amino acid numbers 21 to 129 of the amino acid sequence of SEQ ID NO: 129;

b4) an amino acid sequence having at least 95% identity to any one amino acid sequence selected from the amino acid sequences b1) to b3);

b5) an amino acid sequence having at least 99% identity to any one amino acid sequence selected from the amino acid sequences b1) to b3); and

b6) an amino acid sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several amino acid residues in any one amino acid sequence selected from the amino acid sequences b1) to b3).

43. The antibody according to any one of claims 1 to 42, wherein the antibody is an antibody comprising a heavy chain in which one to several carboxyl-terminal amino acids are deleted.

44. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 43, wherein the antibody is an antibody having a pyroglutamylated amino-terminal amino acid residue in a heavy or light chain thereof.

45. A pharmaceutical composition comprising at least one antibody or antigen-binding fragment according to any one of claims 1 to 44.

46. The pharmaceutical composition according to claim 45, wherein the pharmaceutical composition is a therapeutic and/or prophylactic drug for ectopic ossification.

47. A pharmaceutical composition for the treatment and/or prevention of ectopic ossification, comprising at least any one antibody or antigen-binding fragment thereof according to any one of claims 1 to 44 and at least any one drug selected from the group consisting of anti-inflammatory drugs, steroids, bisphosphonates, muscle relaxants, and retinoic acid receptor (RAR) γ agonists.

48. The pharmaceutical composition according to claim 46 or 47, wherein the ectopic ossification is fibrodysplasia ossificans progressiva (FOP), progressive osseous heteroplasia (POH), traumatic ectopic ossification, or ectopic ossification after implant arthroplasty.

49. The pharmaceutical composition according to claim 46 or 47, wherein the ectopic ossification is fibrodysplasia ossificans progressiva (FOP).

50. The pharmaceutical composition according to claim 45, wherein the pharmaceutical composition is a therapeutic and/or prophylactic drug for anemia.

51. The pharmaceutical composition according to claim 45, wherein the pharmaceutical composition is a therapeutic and/or prophylactic drug for diffuse intrinsic pontine glioma (DIPG).

52. A method for treating and/or preventing ectopic ossification, comprising administering at least one antibody or antigen-binding fragment according to any one of claims 1 to 44, or at least one pharmaceutical composition according to any one of claims 45 to 49.

53. A method for treating and/or preventing ectopic ossification, comprising administering at least one antibody or antigen-binding fragment thereof according to any one of claims 1 to 44, or at least one pharmaceutical composition according to any one of claims 45 to 49, and at least any one drug selected from the group consisting of anti-inflammatory drugs, steroids, bisphosphonates, muscle relaxants, and retinoic acid receptor (RAR) γ agonists, concurrently or separately.

54. The method according to claim 52 or 53, wherein the ectopic ossification is fibrodysplasia ossificans progressiva (FOP), progressive osseous heteroplasia (POH), traumatic ectopic ossification, or ectopic ossification after implant arthroplasty.

55. The method according to claim 52 or 53, wherein the ectopic ossification is fibrodysplasia ossificans progressiva (FOP).

56. A method for treating and/or preventing anemia, comprising administering at least one antibody or antigen-binding fragment according to any one of claims 1 to 44, or at least one pharmaceutical composition according to claim 45 or 50.

57. A method for treating and/or preventing diffuse intrinsic pontine glioma (DIPG), comprising administering at least one antibody or antigen-binding fragment according to any one of claims 1 to 44, or at least one pharmaceutical composition according to claim 45 or 51.

58. A polynucleotide encoding an antibody according to any one of claims 1 to 44.

59. A polynucleotide comprising

- (a) a polynucleotide selected from the group consisting of the following nucleotide sequences:
 - a1) a nucleotide sequence consisting of nucleotide numbers 58 to 426 of the nucleotide sequence of SEQ ID NO: 27;
 - a2) a nucleotide sequence consisting of nucleotide numbers 58 to 426 of the nucleotide sequence of SEQ ID NO: 29;
 - a3) a nucleotide sequence consisting of nucleotide numbers 58 to 426 of the nucleotide sequence of SEQ ID NO: 104;
 - a4) a nucleotide sequence having at least 95% identity to any of the nucleotide sequences a1) to a3);

- a5) a nucleotide sequence having at least 99% identity to any of the nucleotide sequences a1) to a3);
- a6) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any of the nucleotide sequences a1) to a3); and
- a7) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in the nucleotide sequence a1) or a2); and/or
- (b) a polynucleotide selected from the group consisting of the following nucleotide sequences:
 - b1) a nucleotide sequence consisting of nucleotide numbers 86 to 424 of the nucleotide sequence of SEQ ID NO: 31;
 - b2) a nucleotide sequence consisting of nucleotide numbers 86 to 424 of the nucleotide sequence of SEQ ID NO: 33;
 - b3) a nucleotide sequence consisting of nucleotide numbers 86 to 424 of the nucleotide sequence of SEQ ID NO: 35;
 - b4) a nucleotide sequence consisting of nucleotide numbers 86 to 424 of the nucleotide sequence of SEQ ID NO: 37;
 - b5) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b4);
 - b6) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b4);
 - b7) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences b1) to b4); and
 - b8) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences b1) to b4).

60. A polynucleotide comprising

- (a) a polynucleotide selected from the group consisting of the following nucleotide sequences:

- a1) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 39;
- a2) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 41;
- a3) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 43;
- a4) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 45;
- a5) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 47;
- a6) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 106;
- a7) a nucleotide sequence consisting of nucleotide numbers 58 to 420 of the nucleotide sequence of SEQ ID NO: 108;
- a8) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences a1) to a7);
- a9) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences a1) to a7);
- a10) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences a1) to a7); and
- a11) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences a1) to a7); and/or
- (b) a polynucleotide selected from the group consisting of the following nucleotide sequences:
 - b1) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 49;
 - b2) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 51;

- b3) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 53;
- b4) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 55;
- b5) a nucleotide sequence consisting of nucleotide numbers 86 to 412 of the nucleotide sequence of SEQ ID NO: 57;
- b6) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b5);
- b7) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b5);
- b8) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences b1) to b5); and
- b9) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences b1) to b5).

61. A polynucleotide comprising

- (a) a polynucleotide selected from the group consisting of the following nucleotide sequences:
 - a1) a nucleotide sequence consisting of nucleotide numbers 58 to 411 of the nucleotide sequence of SEQ ID NO: 110;
 - a2) a nucleotide sequence consisting of nucleotide numbers 58 to 411 of the nucleotide sequence of SEQ ID NO: 112;
 - a3) a nucleotide sequence having at least 95% identity to the nucleotide sequence a1) or a2);
 - a4) a nucleotide sequence having at least 99% identity to the nucleotide sequence a1) or a2);
 - a5) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to the nucleotide sequence a1) or a2); and
 - a6) a nucleotide sequence comprising a substitution(s), a deletion (s), or an addition(s) of one to several nucleotides in the nucleotide sequence a1) or a2); and/or

- (b) a polynucleotide selected from the group consisting of the following nucleotide sequences:
- b1) a nucleotide sequence consisting of nucleotide numbers 61 to 384 of the nucleotide sequence of SEQ ID NO: 114;
 - b2) a nucleotide sequence consisting of nucleotide numbers 61 to 384 of the nucleotide sequence of SEQ ID NO: 116;
 - b3) a nucleotide sequence consisting of nucleotide numbers 61 to 384 of the nucleotide sequence of SEQ ID NO: 118;
 - b4) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b3);
 - b5) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b3);
 - b6) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences b1) to b3); and
 - b7) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences b1) to b3).

62. A polynucleotide comprising

- (a) a polynucleotide selected from the group consisting of the following nucleotide sequences:
- a1) a nucleotide sequence consisting of nucleotide numbers 58 to 411 of a nucleotide sequence of SEQ ID NO: 120;
 - a2) a nucleotide sequence consisting of nucleotide numbers 58 to 411 of a nucleotide sequence of SEQ ID NO: 122;
 - a3) a nucleotide sequence having at least 95% identity to the nucleotide sequence a1) or a2);
 - a4) a nucleotide sequence having at least 99% identity to the nucleotide sequence a1) or a2);
 - a5) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to the nucleotide sequence a1) or a2); and

a6) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in the nucleotide sequence a1) or a2); and/or

(b) a polynucleotide selected from the group consisting of the following nucleotide sequences:

b1) a nucleotide sequence consisting of nucleotide numbers 61 to 387 of the nucleotide sequence of SEQ ID NO: 124;

b2) a nucleotide sequence consisting of nucleotide numbers 61 to 387 of the nucleotide sequence of SEQ ID NO: 126;

b3) a nucleotide sequence consisting of nucleotide numbers 61 to 387 of the nucleotide sequence of SEQ ID NO: 128;

b4) a nucleotide sequence having at least 95% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b3);

b5) a nucleotide sequence having at least 99% identity to any one nucleotide sequence selected from the nucleotide sequences b1) to b3);

b6) a nucleotide sequence carried by a polynucleotide hybridizing under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to any one nucleotide sequence selected from the nucleotide sequences b1) to b3); and

b7) a nucleotide sequence comprising a substitution(s), a deletion(s), or an addition(s) of one to several nucleotides in any one nucleotide sequence selected from the nucleotide sequences b1) to b3).

63. A vector comprising any one polynucleotide according to any one of claims 58 to 62.

64. A transformed host cell comprising any one polynucleotide according to any one of claims 58 to 62.

65. A transformed host cell comprising a vector according to claim 63.

66. A method for producing an antibody according to any one of claims 1 to 44, comprising the step of culturing a host cell according to claim 64 or 65 and purifying the antibody from the culture product.

FIG. 1

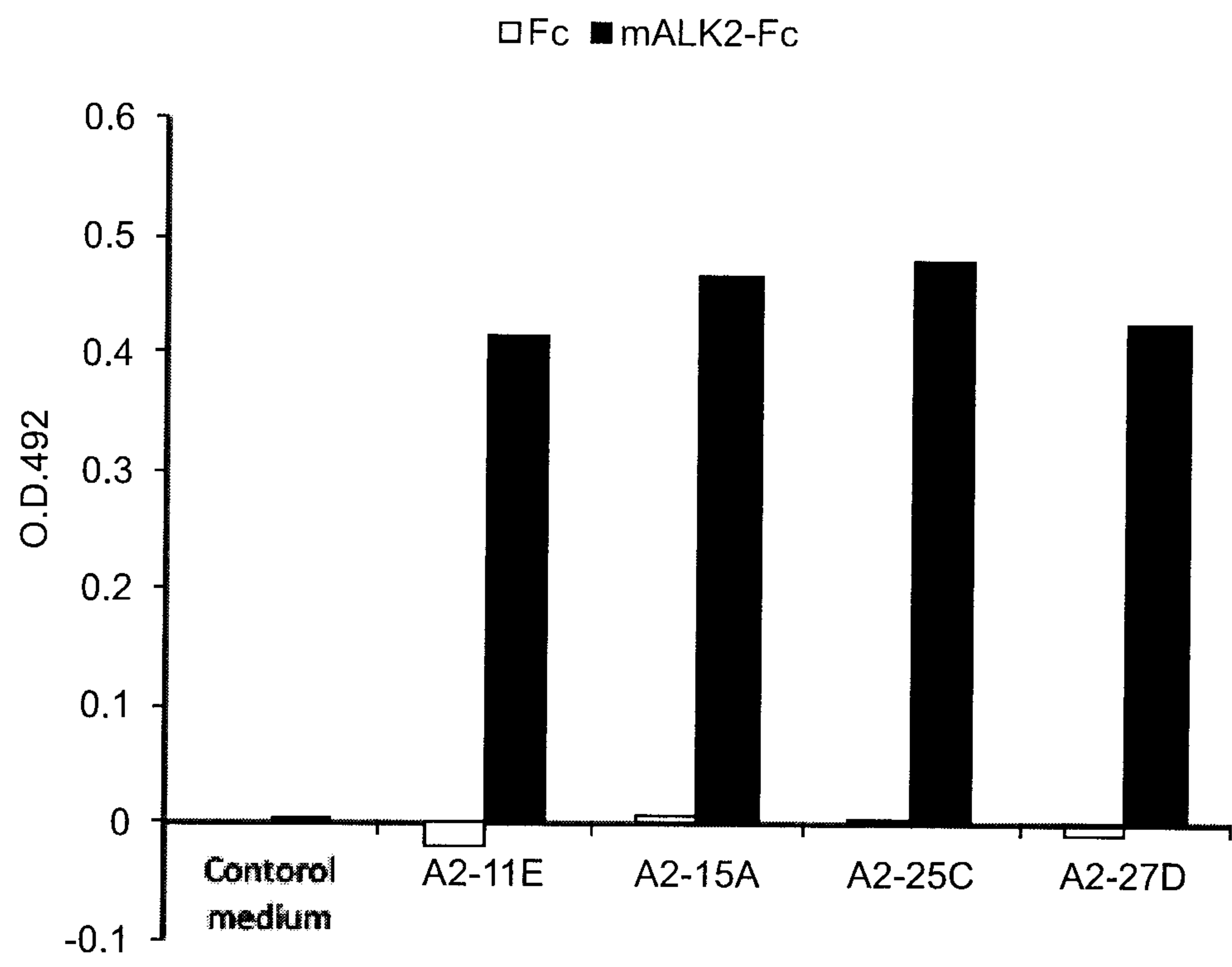


FIG. 2A

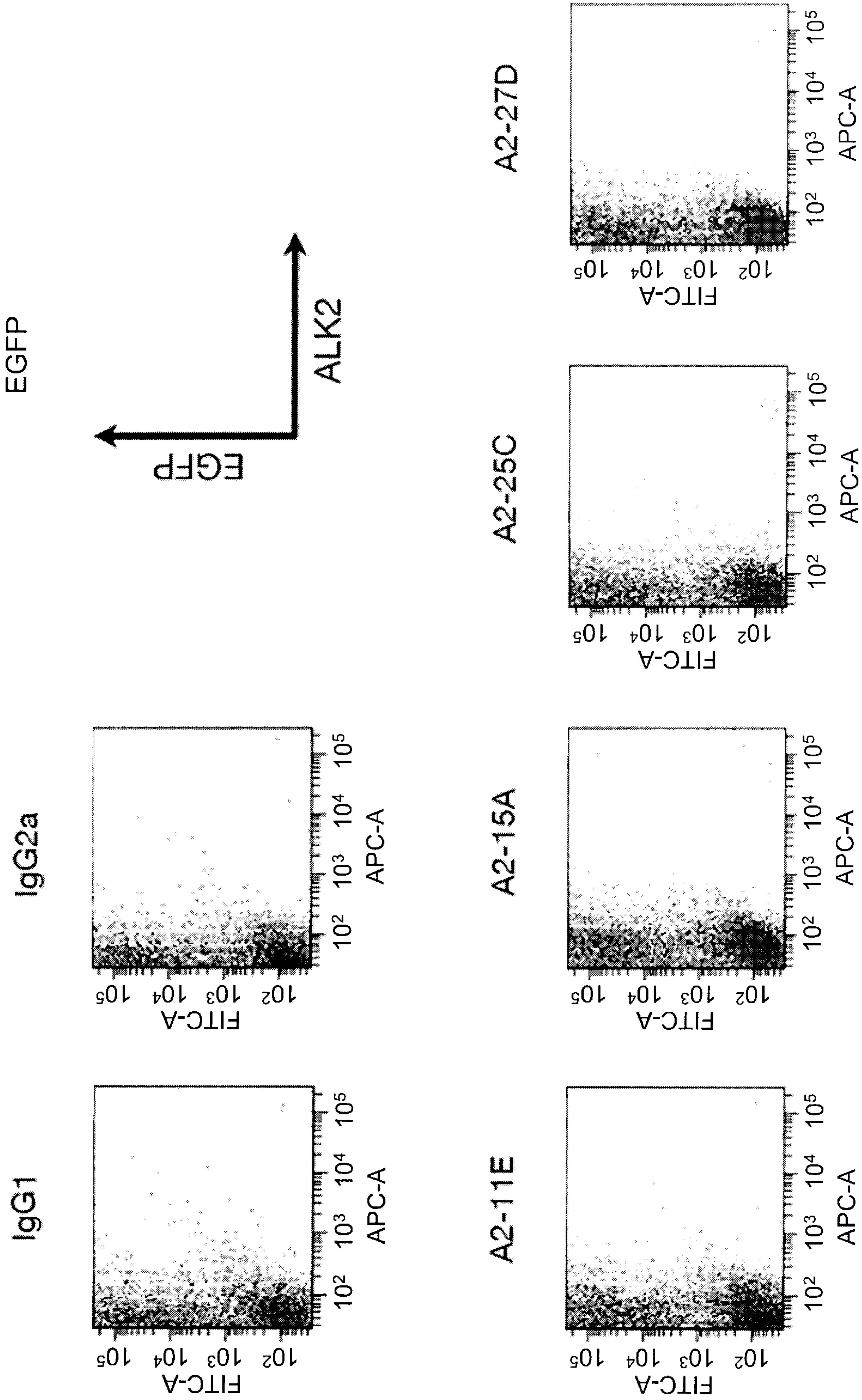


FIG. 2B

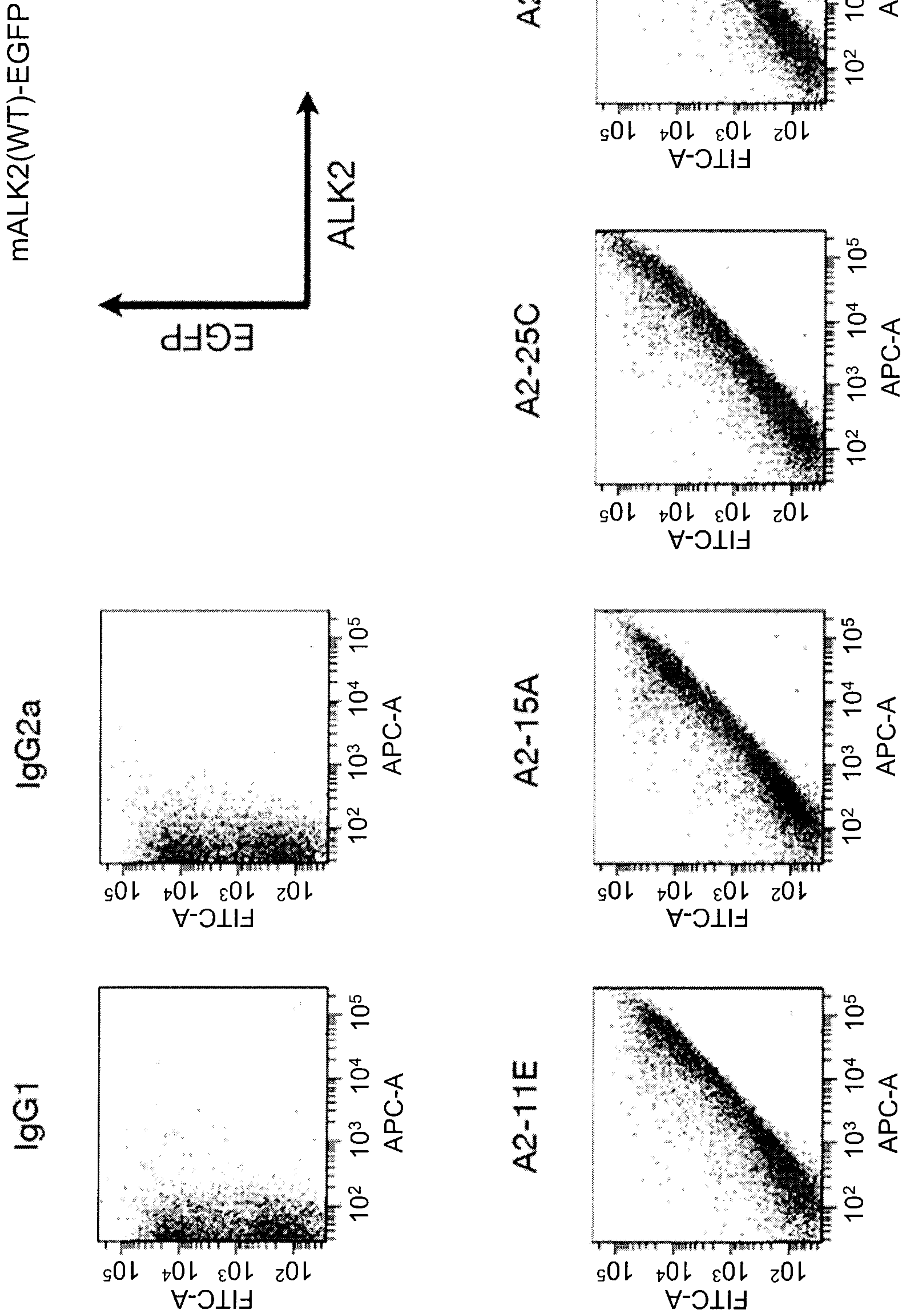
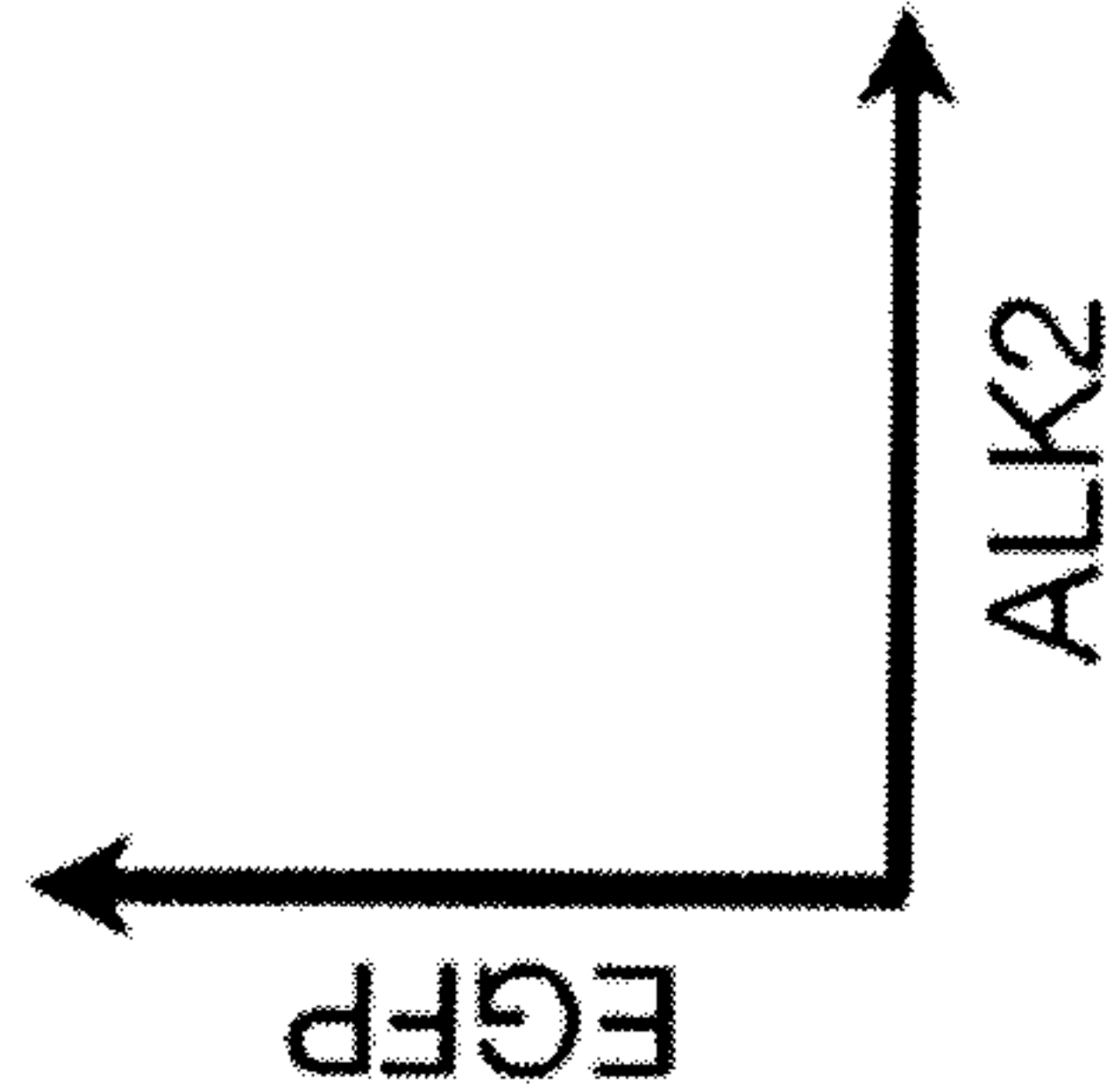
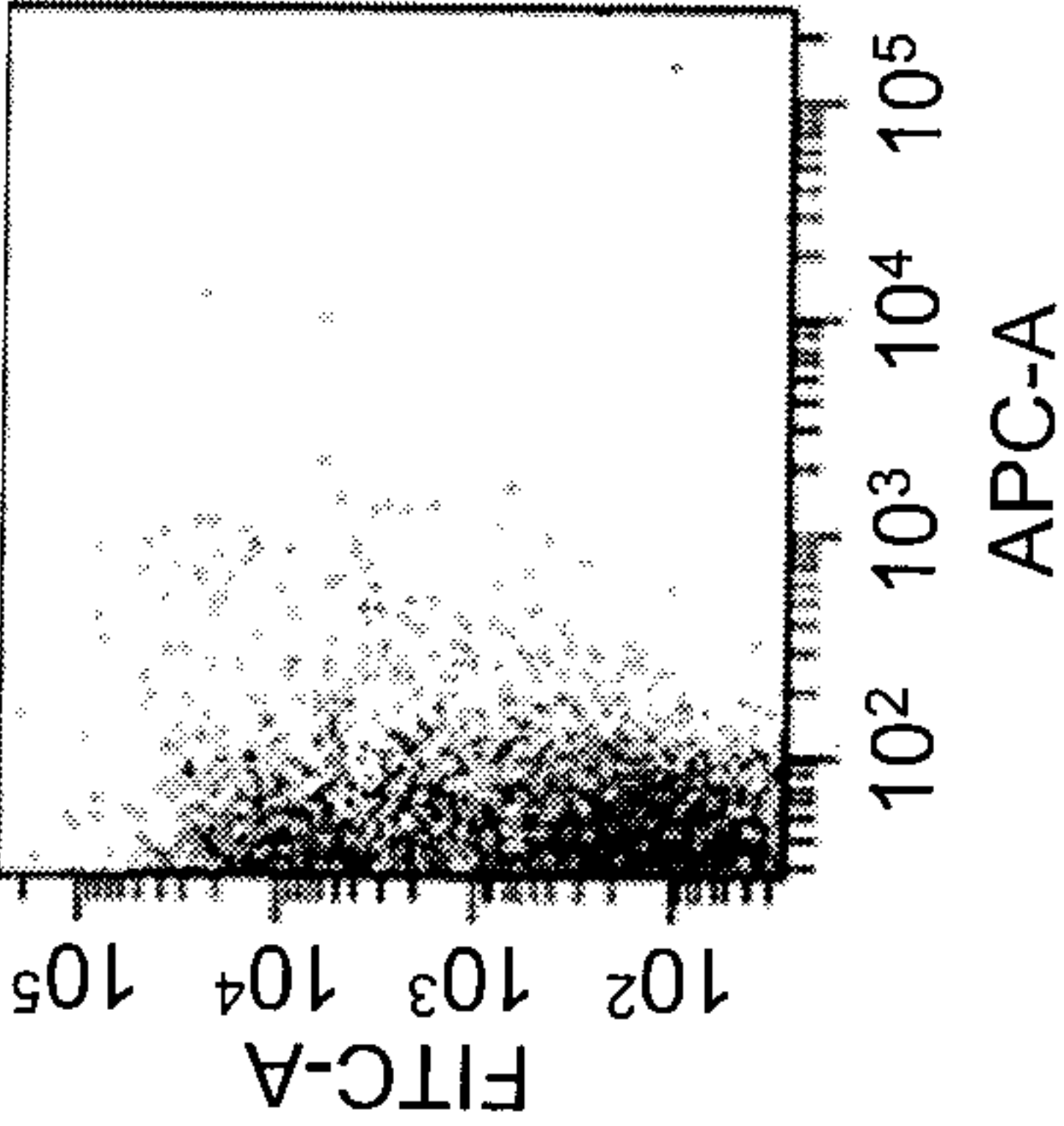


FIG. 2C

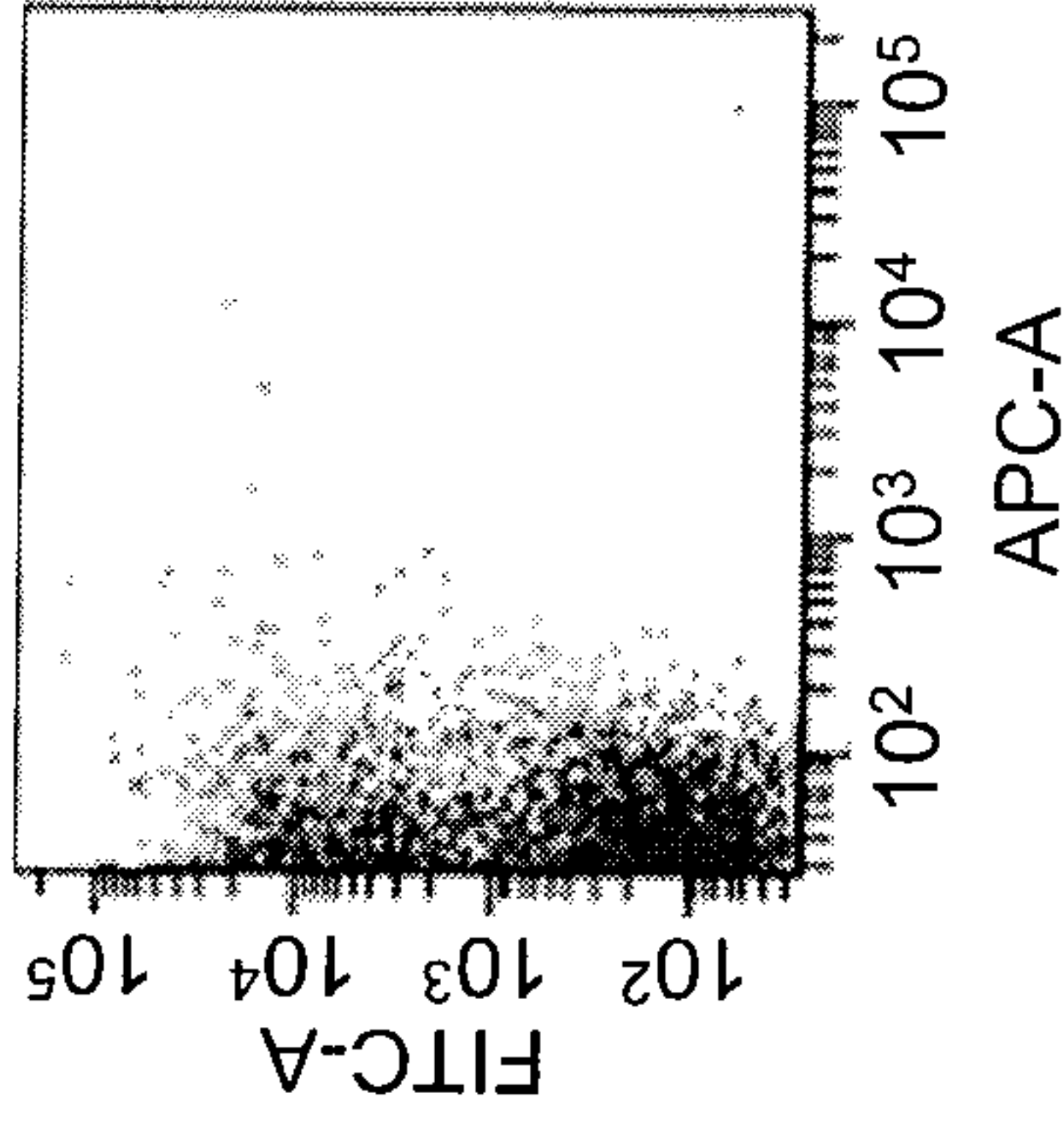
hALK2(WT)-EGFP



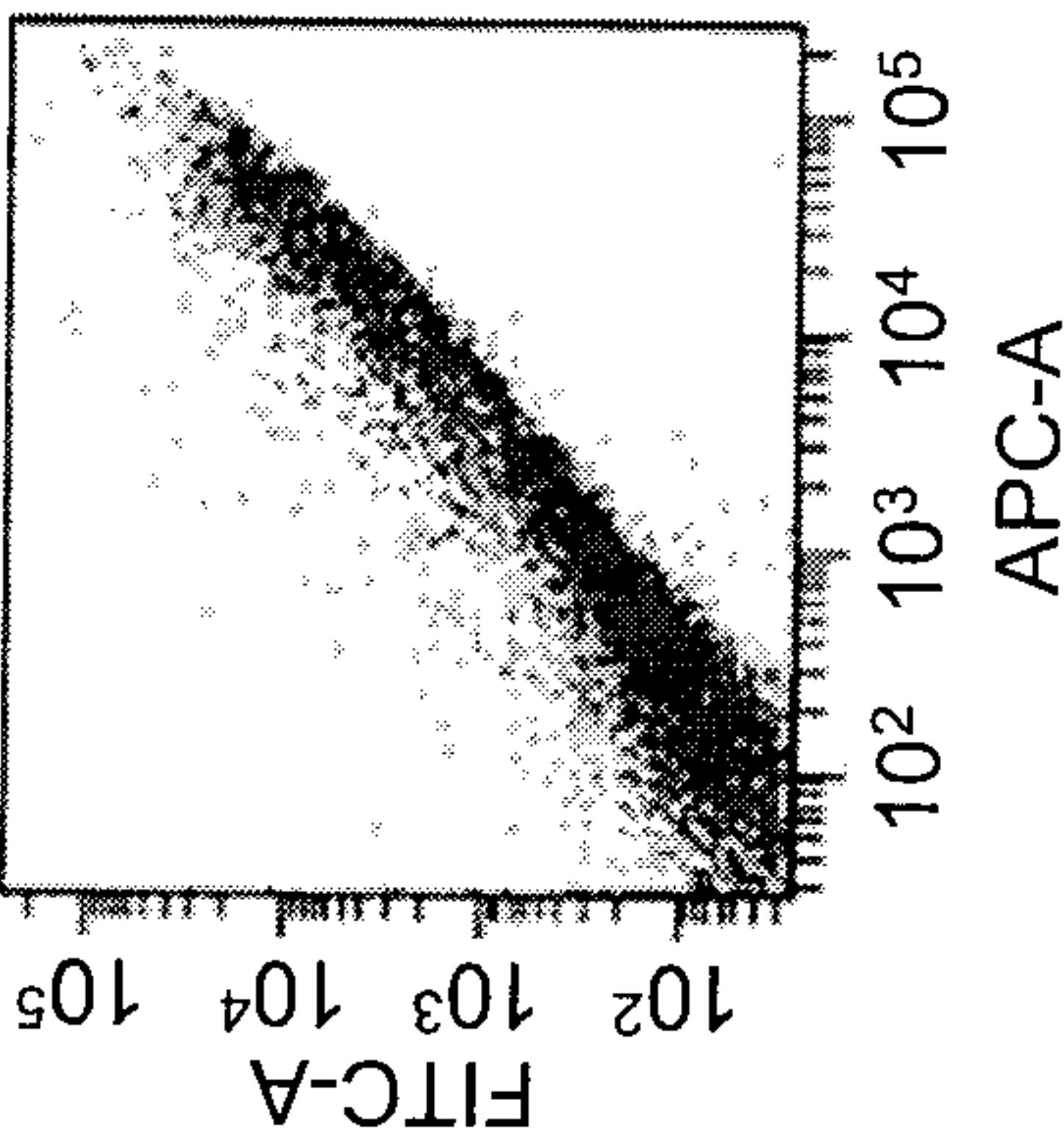
IgG1



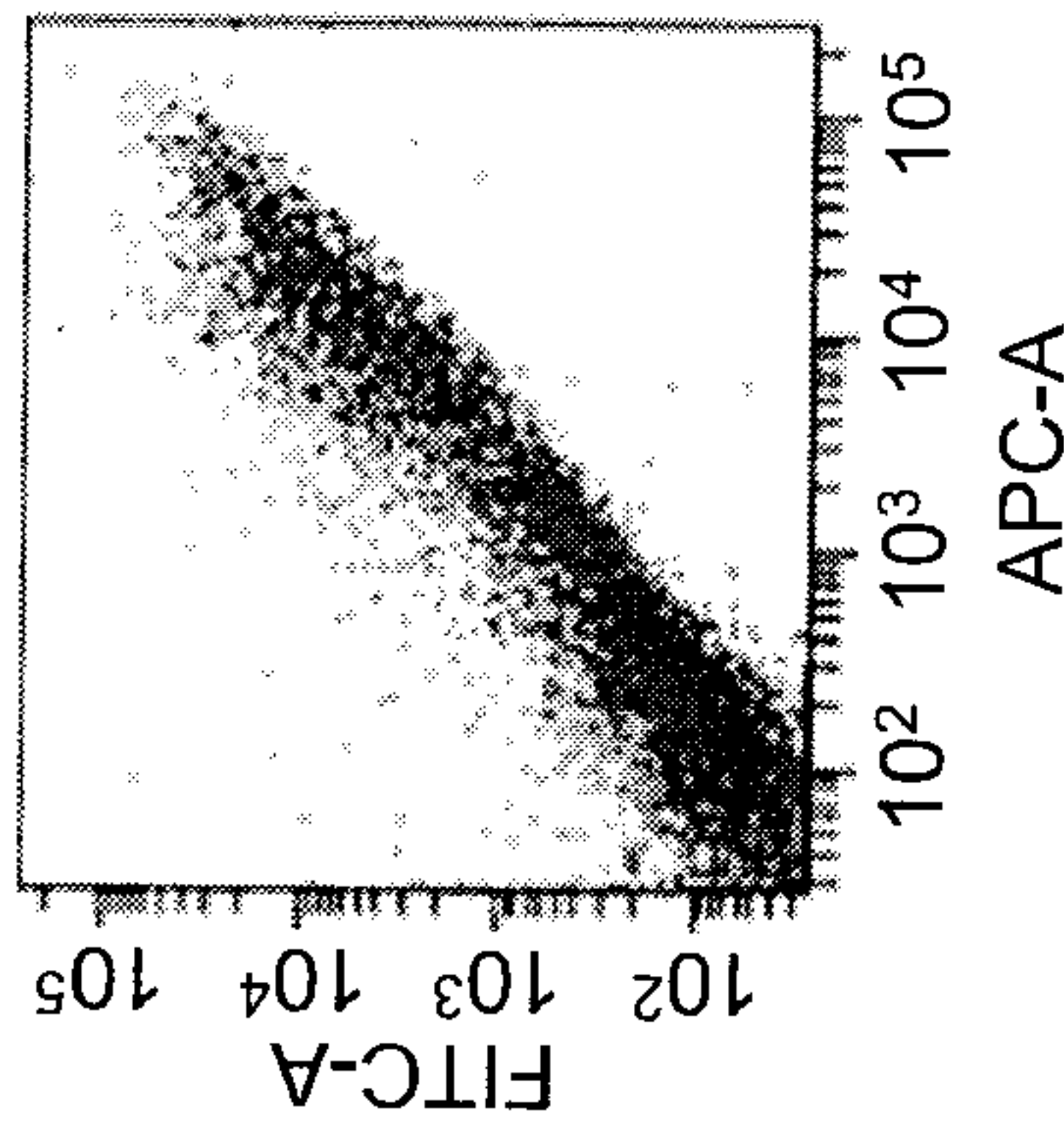
IgG2a



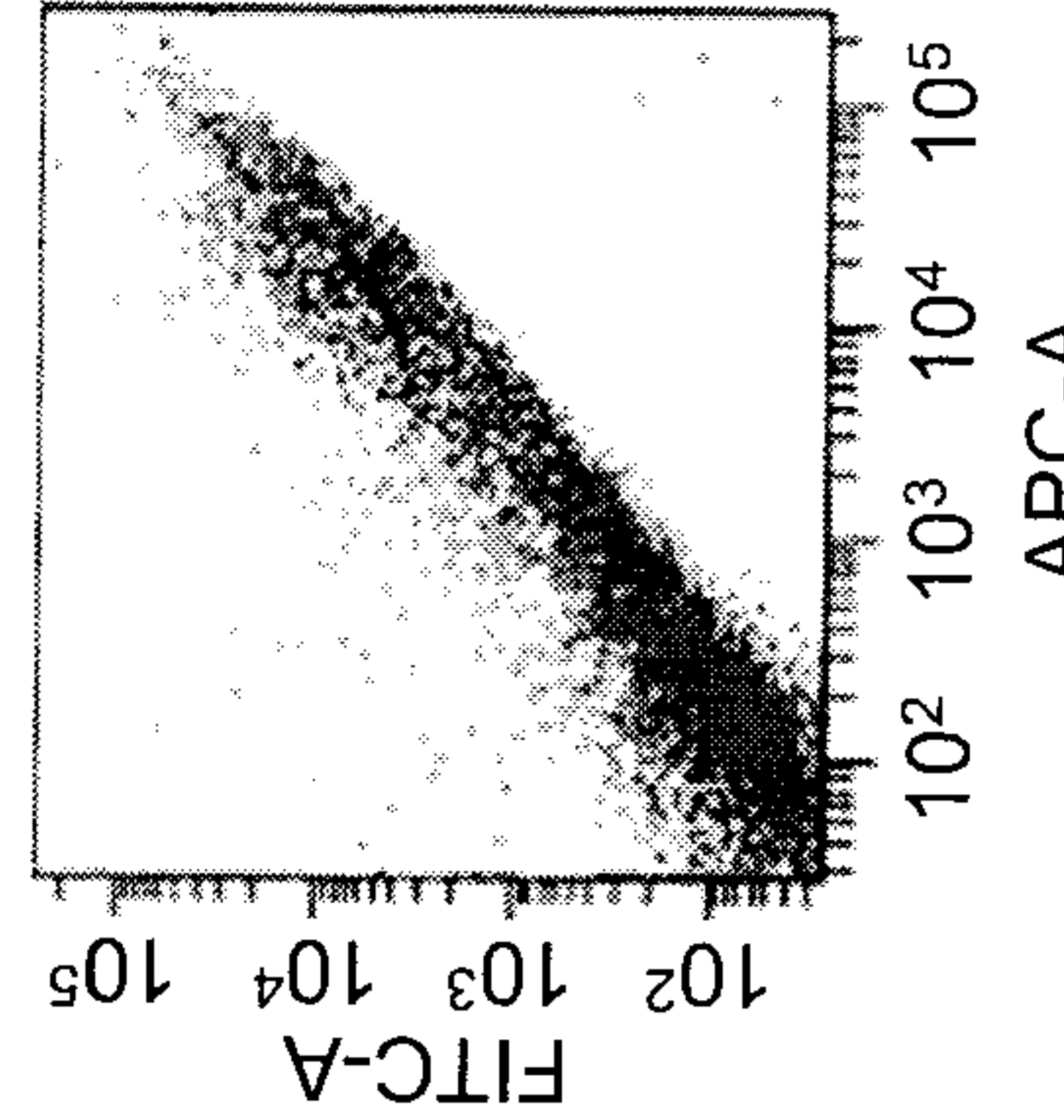
A2-11E



A2-15A



A2-25C



A2-27D

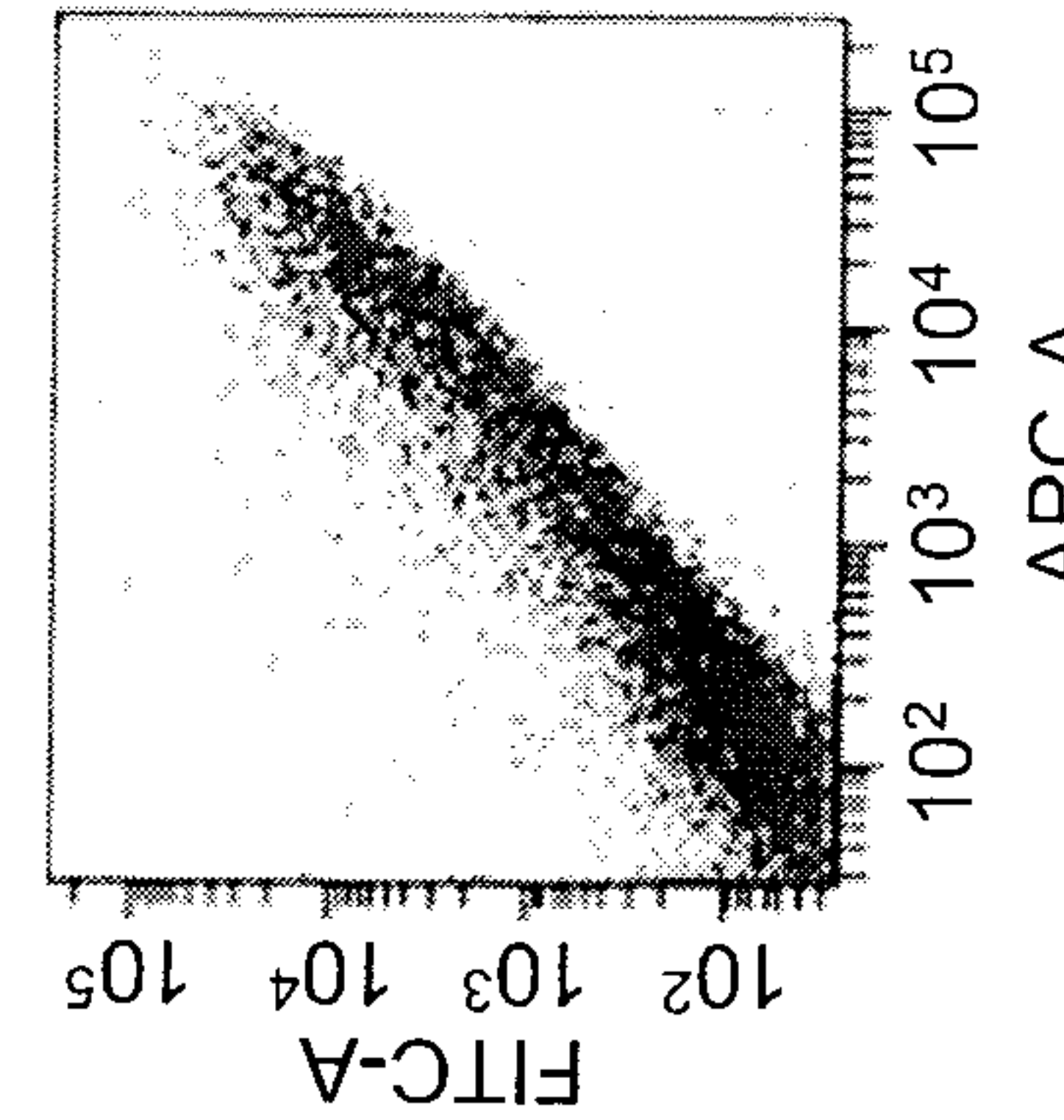
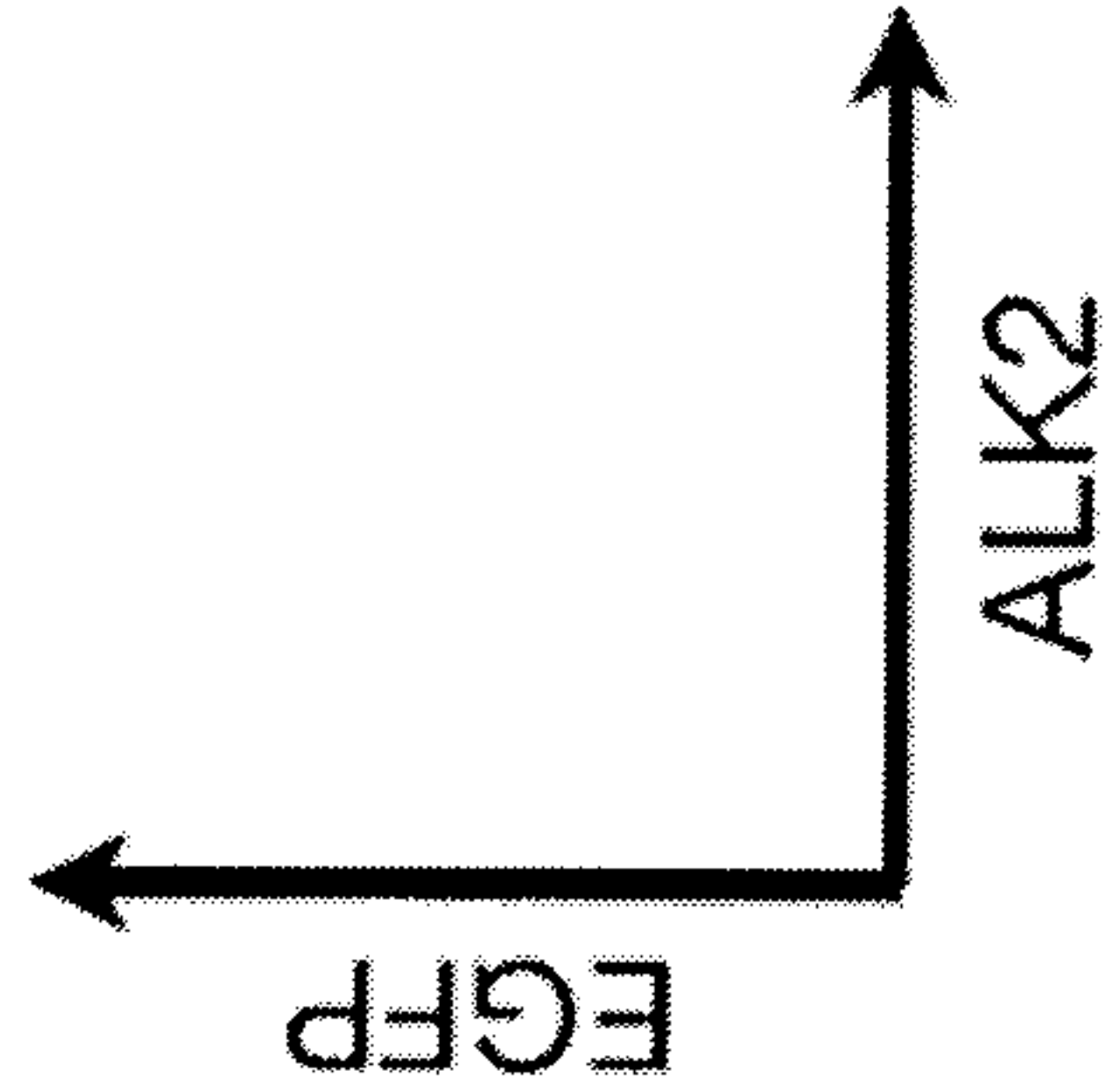
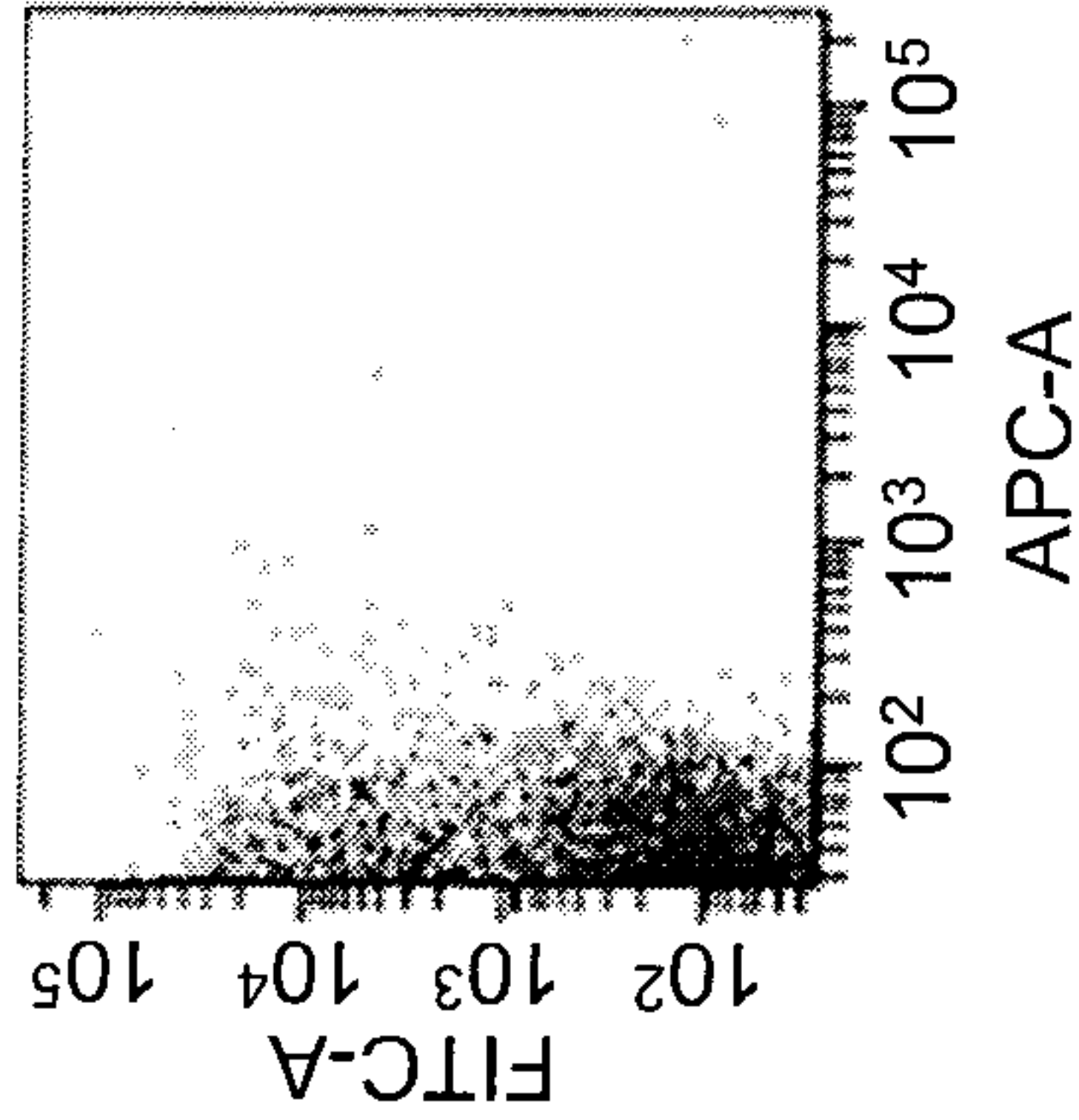


FIG. 2D

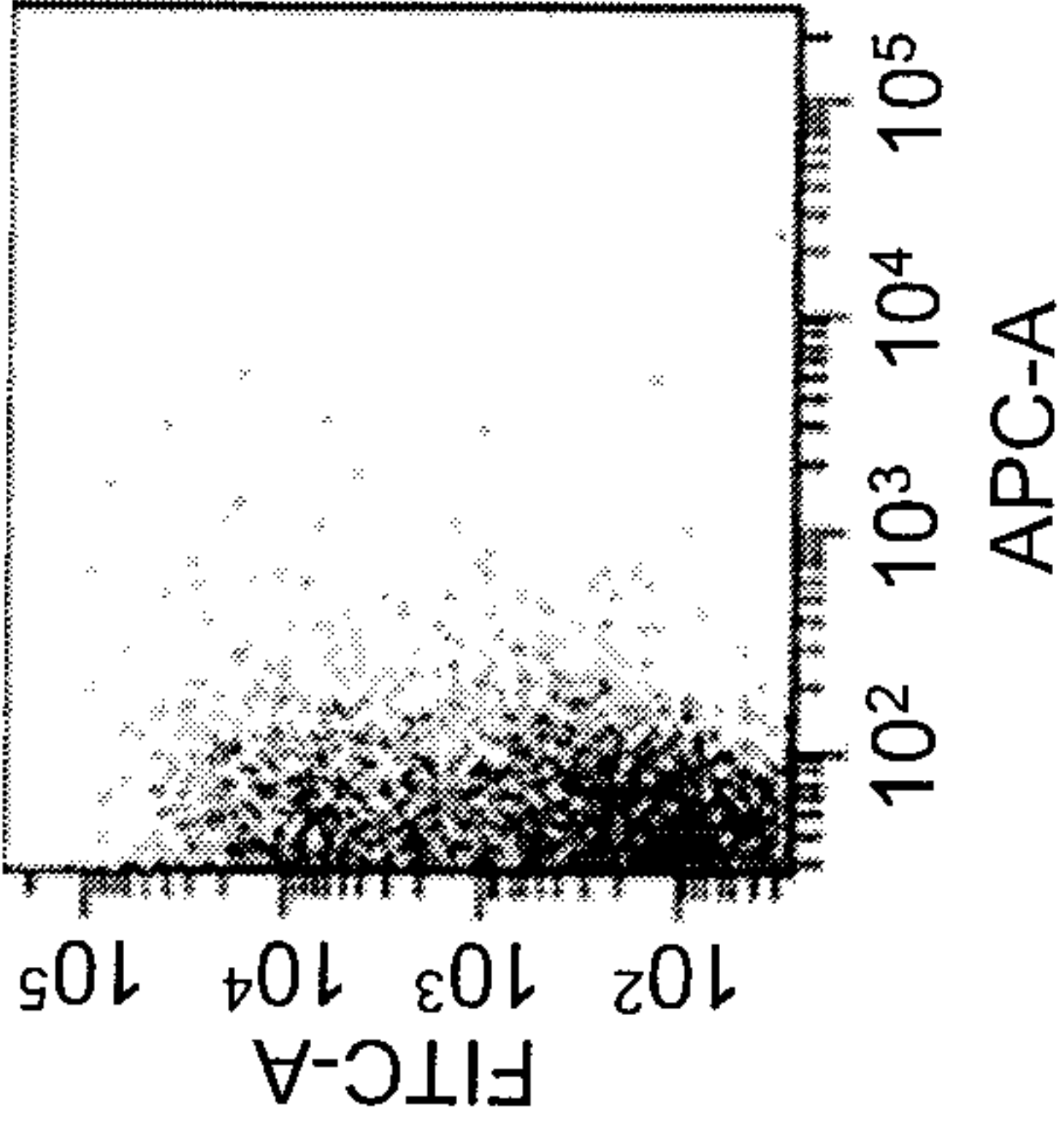
hALK2(R206H)-EGFP



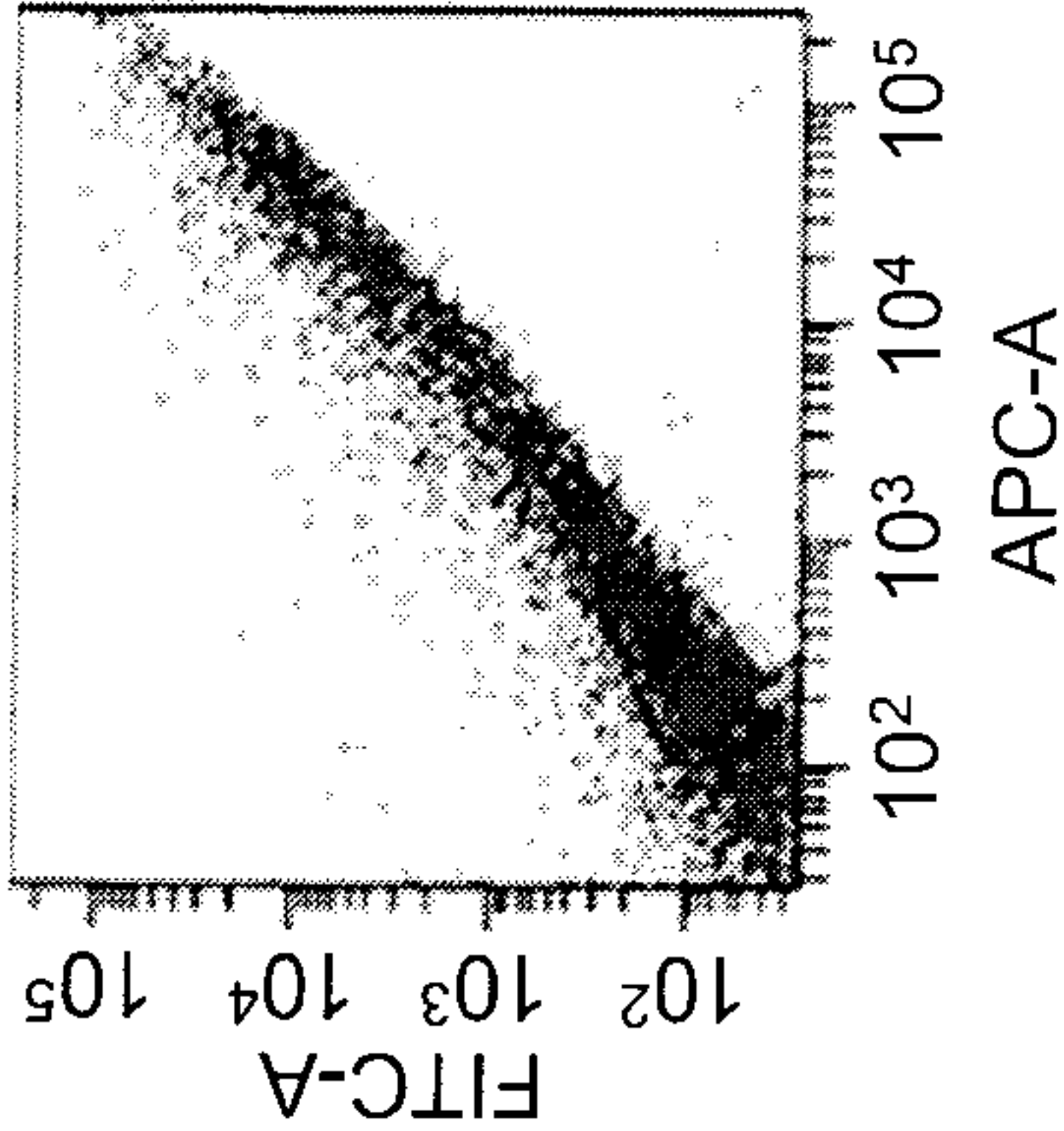
IgG2a



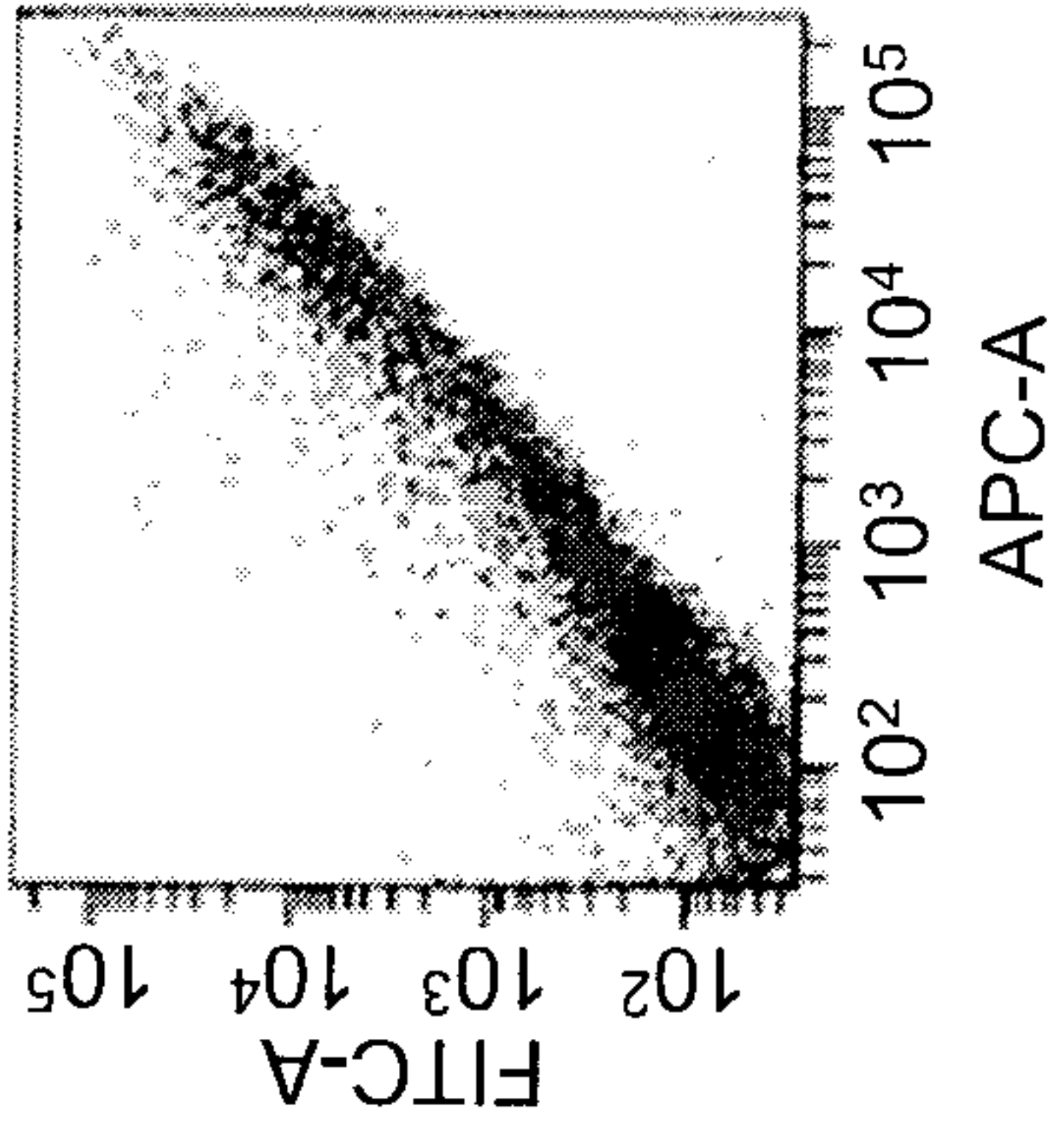
IgG1



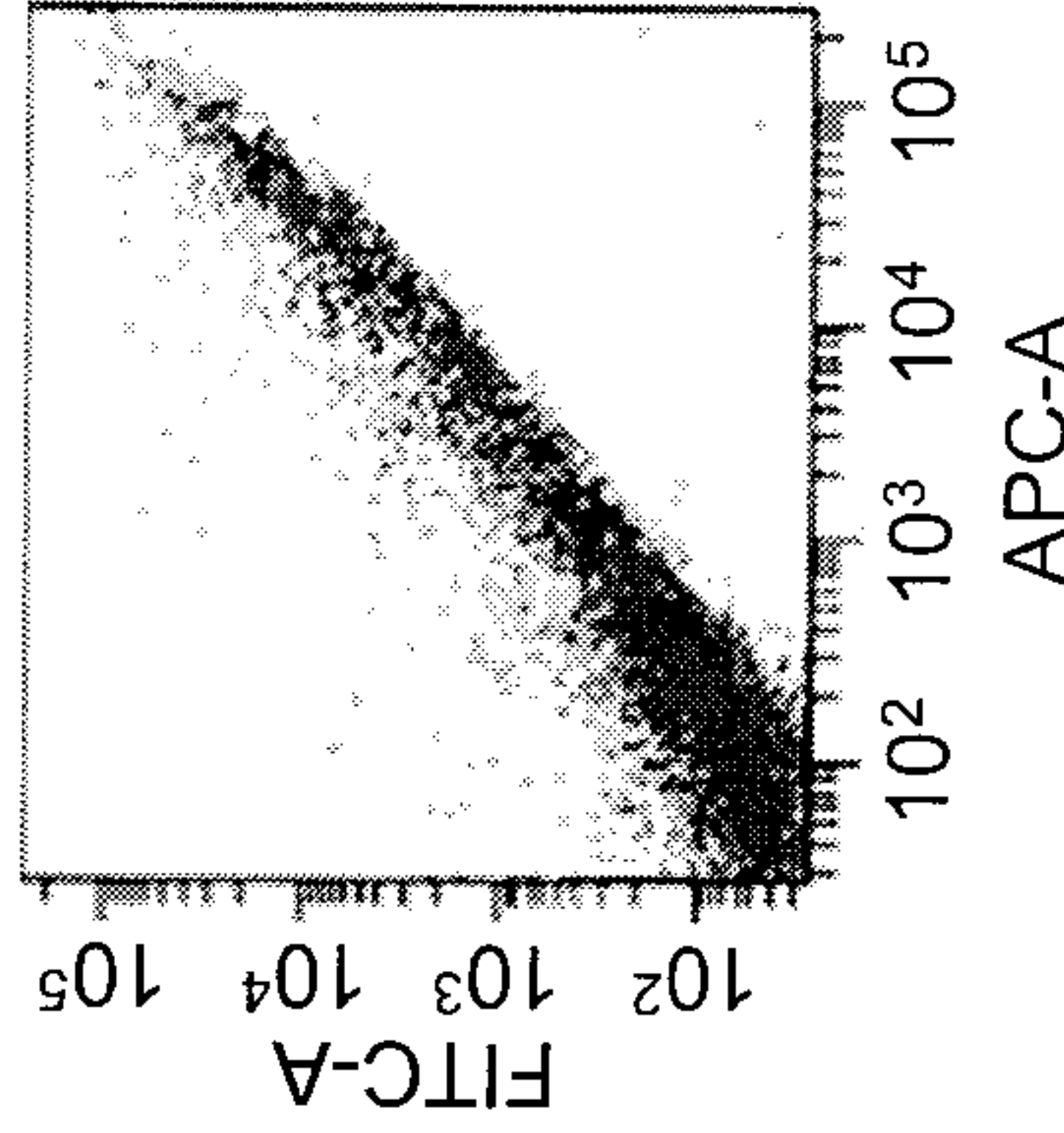
A2-11E



A2-15A



A2-25C



A2-27D

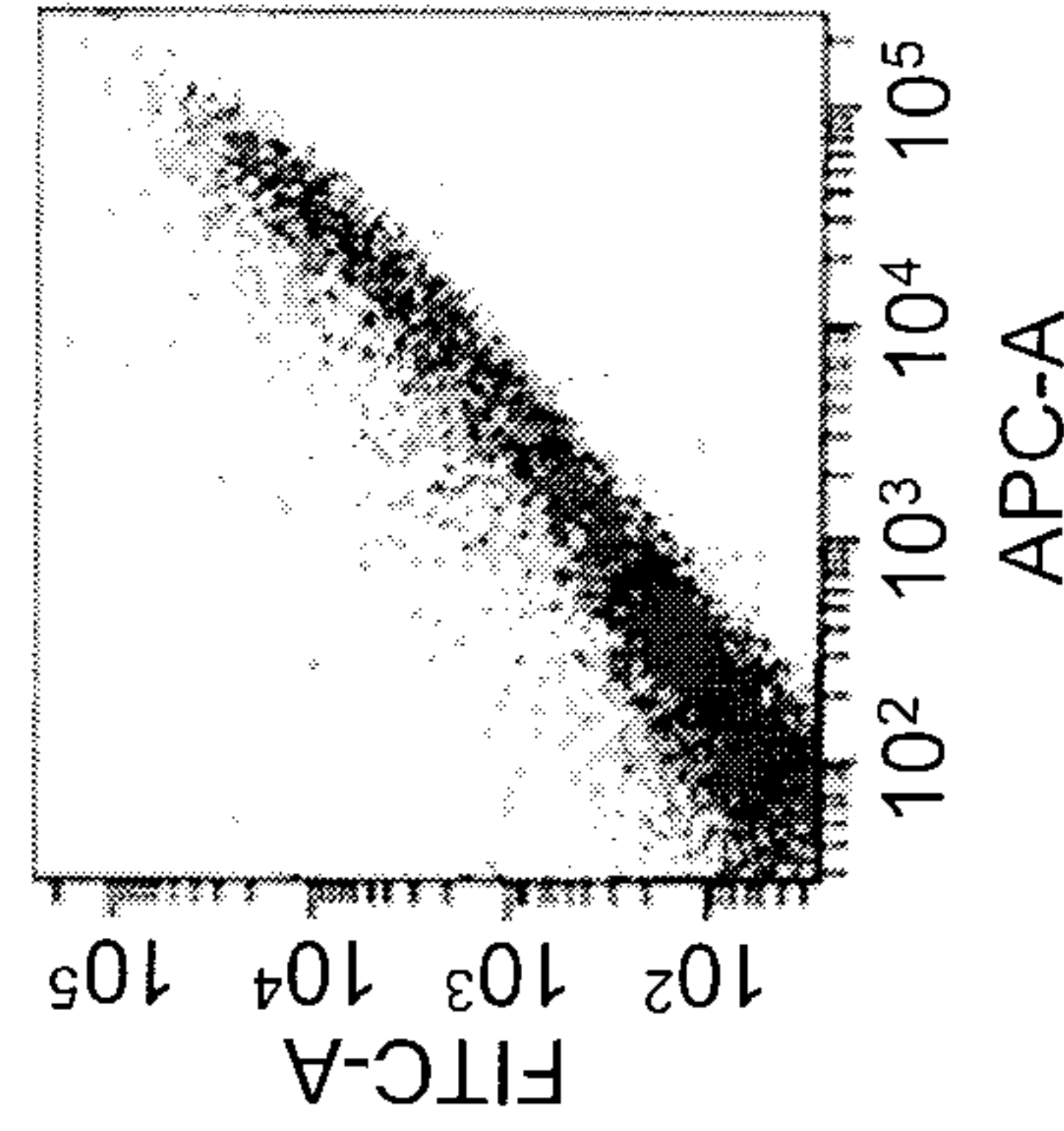


FIG. 3

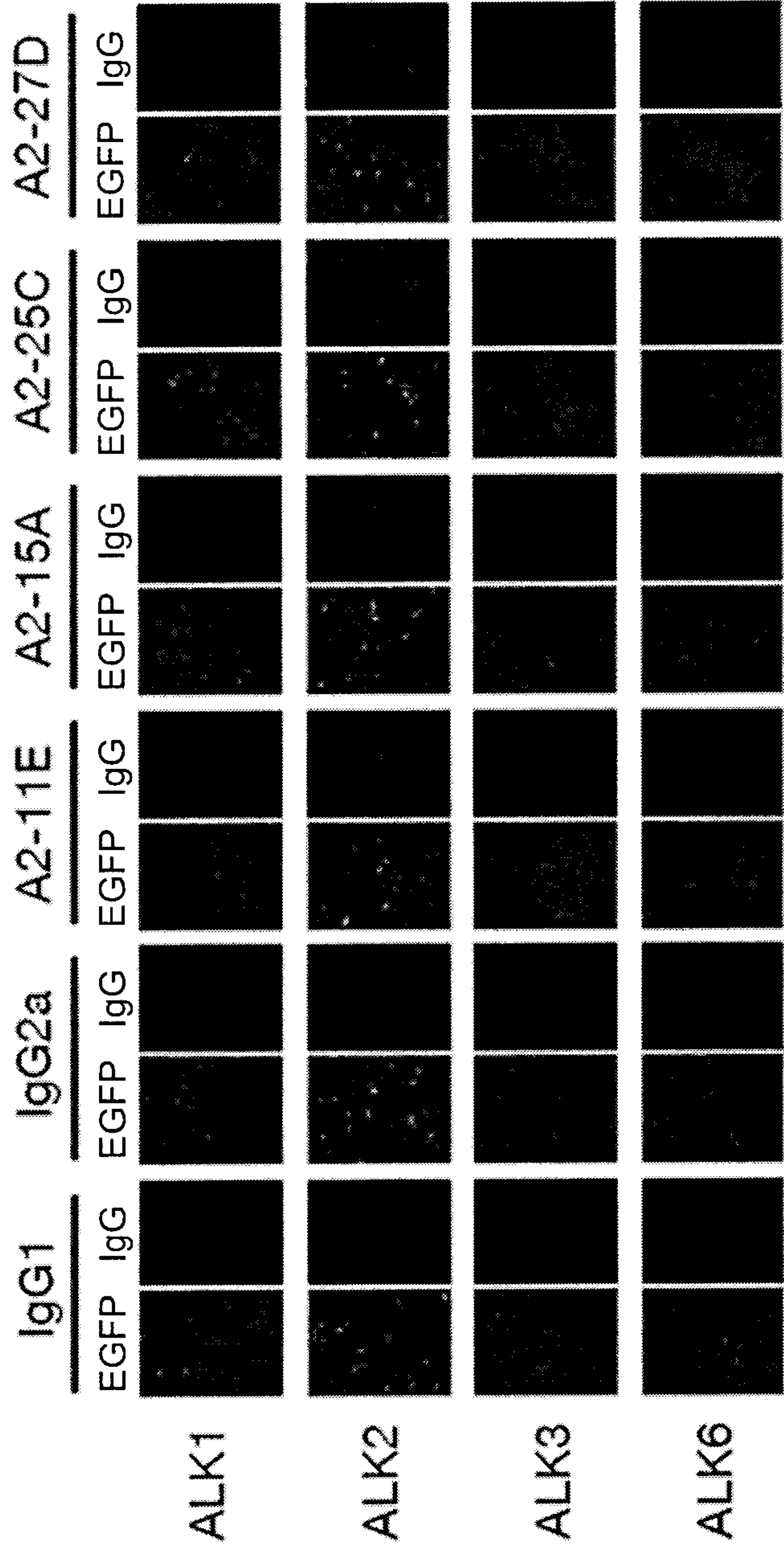


FIG. 4

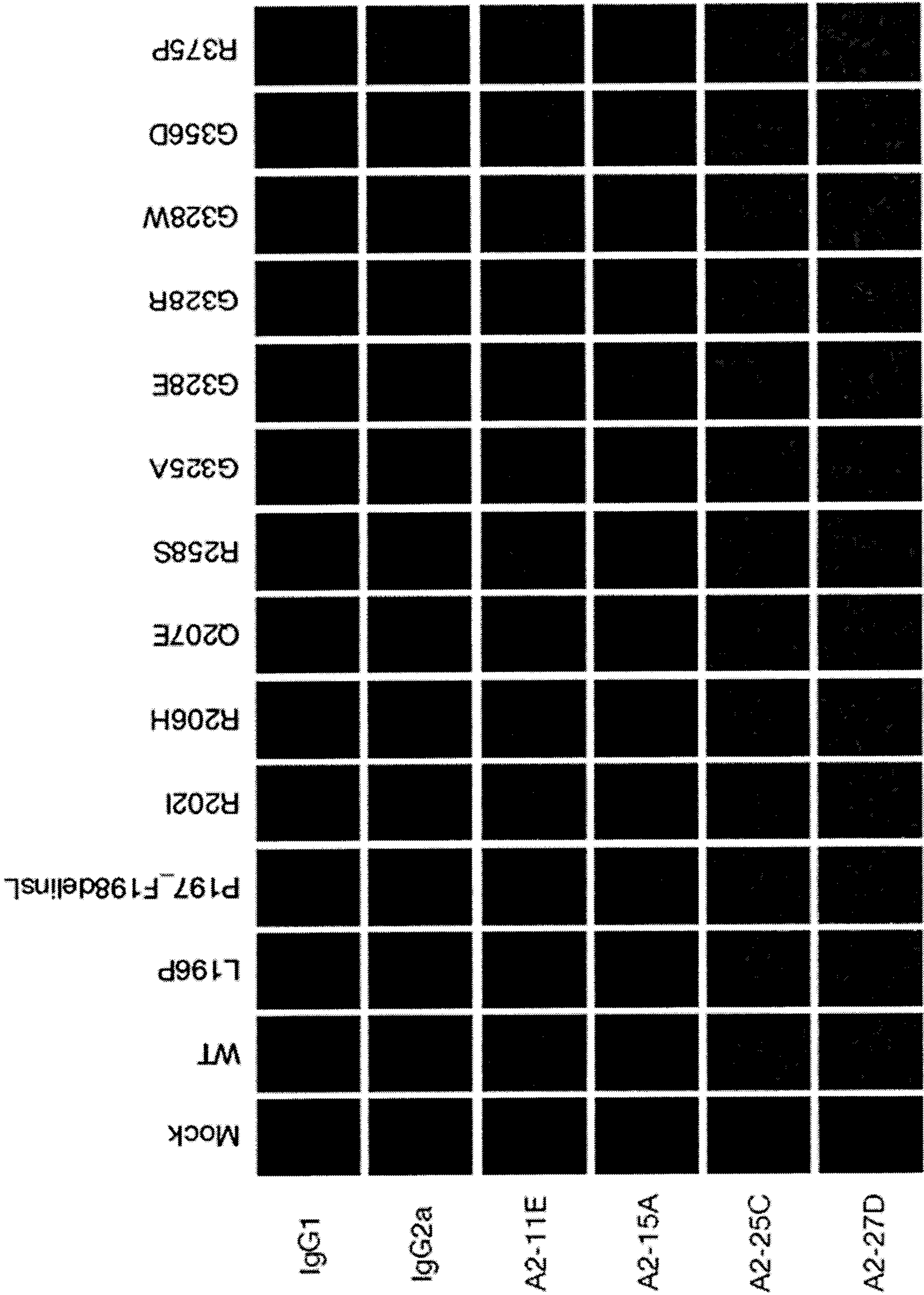


FIG. 5

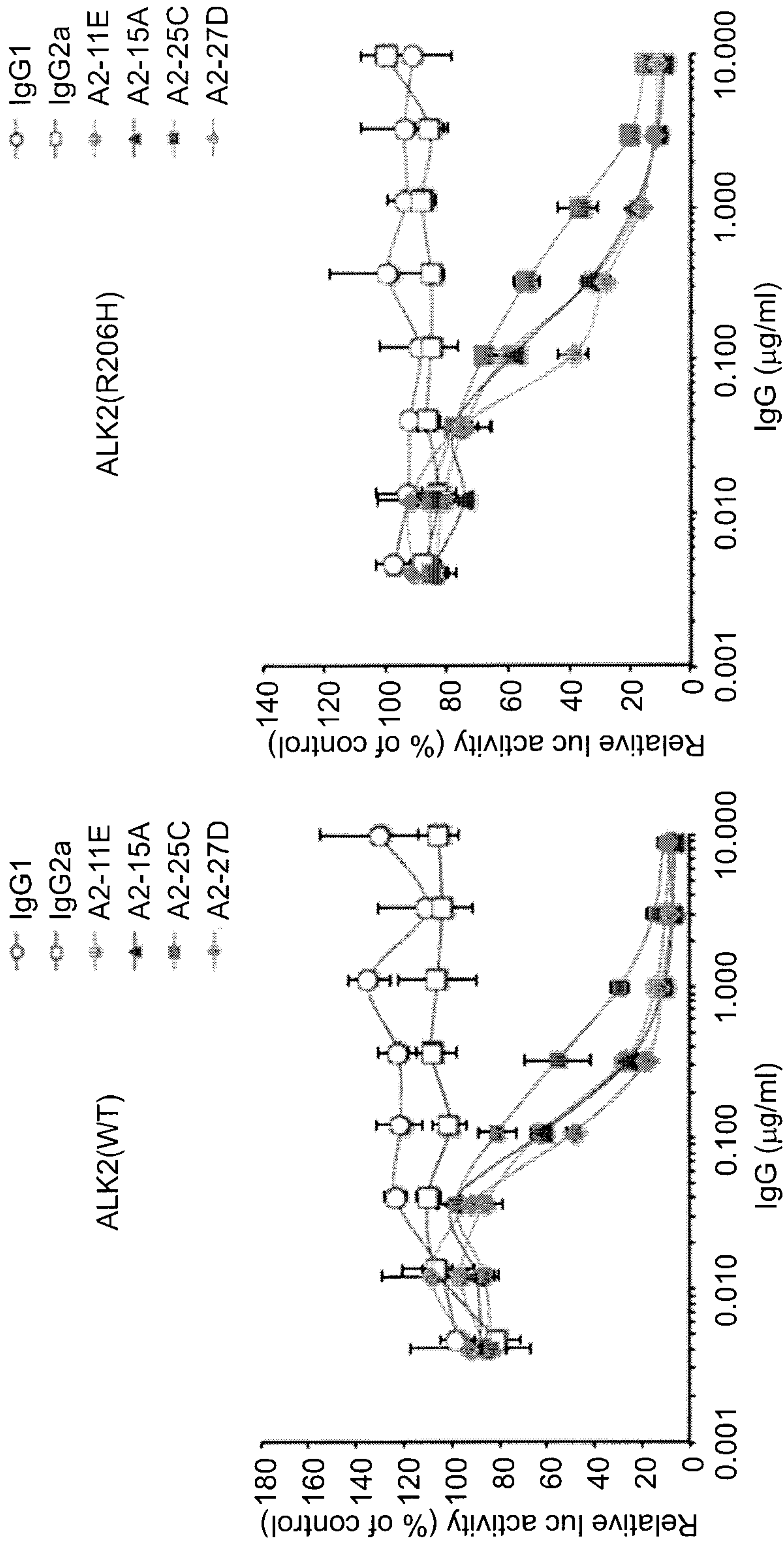


FIG. 6

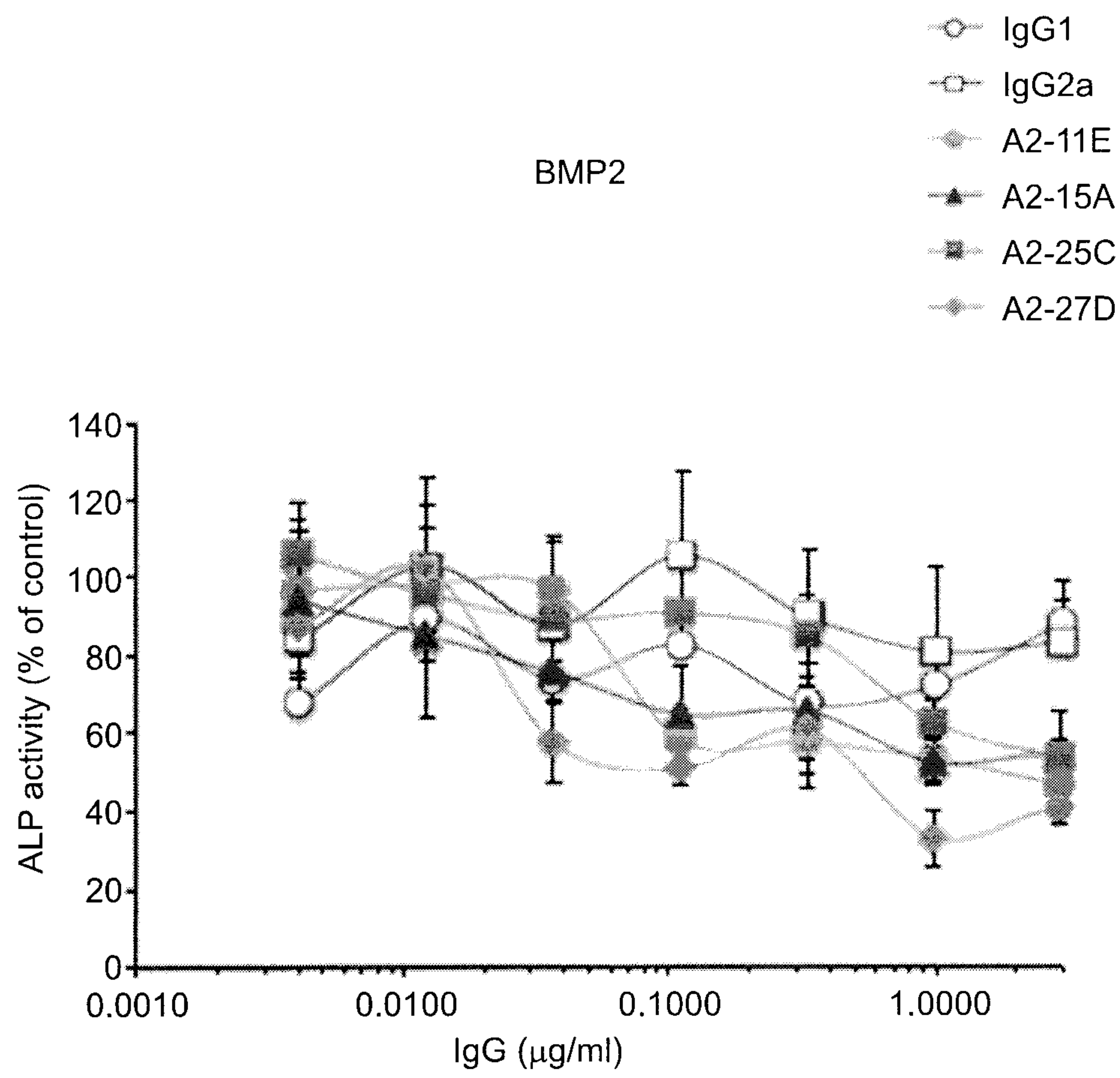


FIG. 7

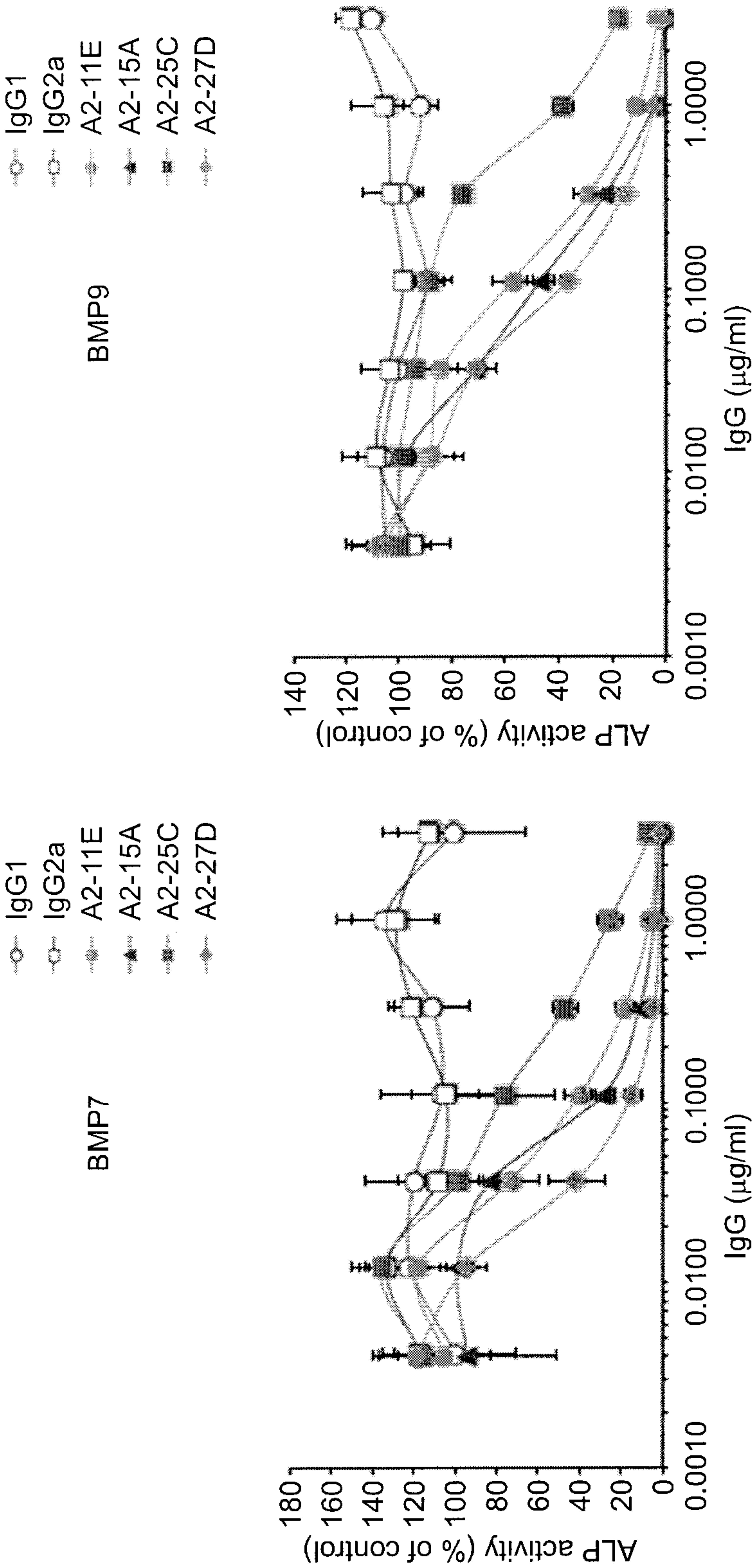


FIG. 8

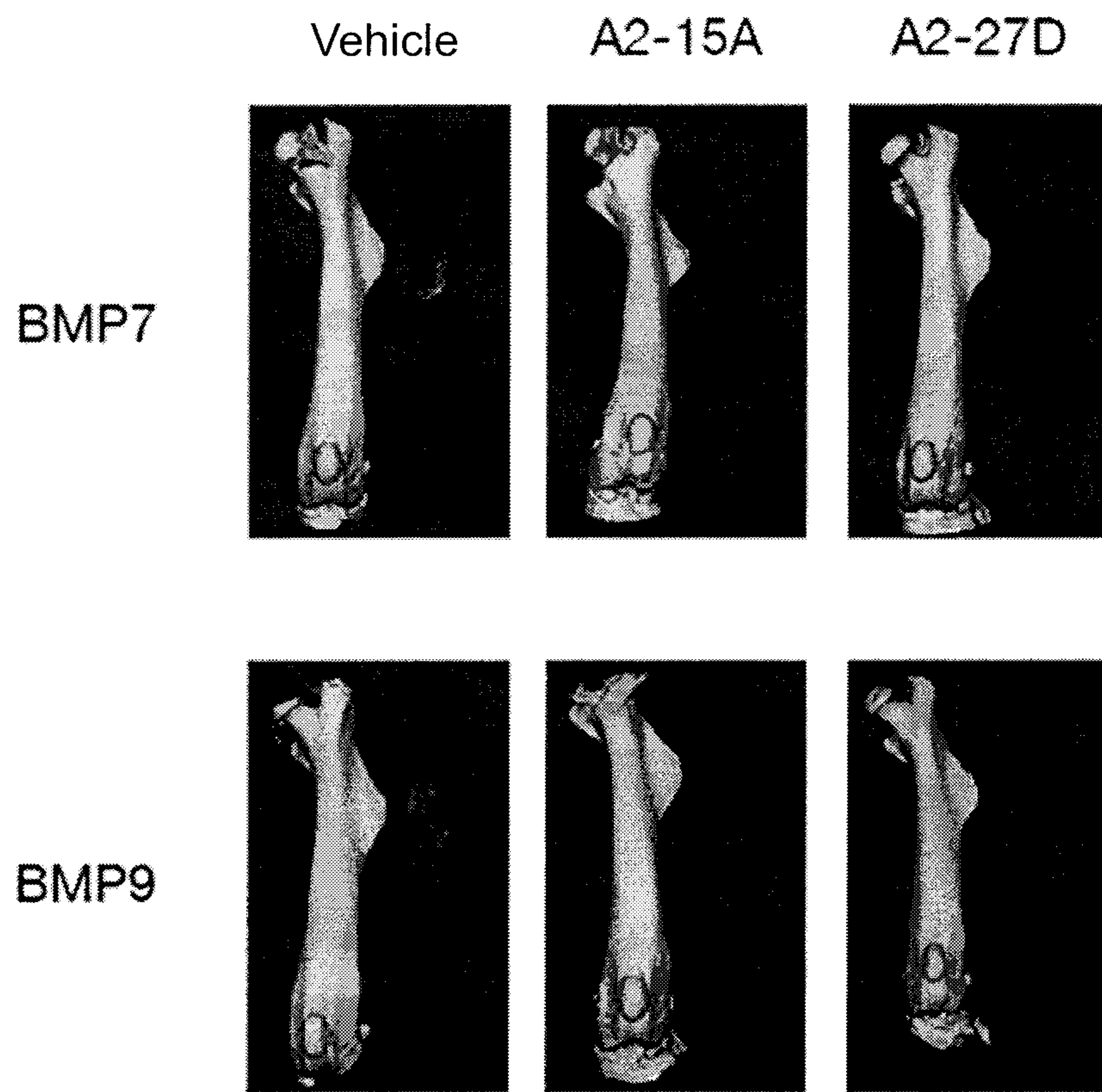


Fig. 9

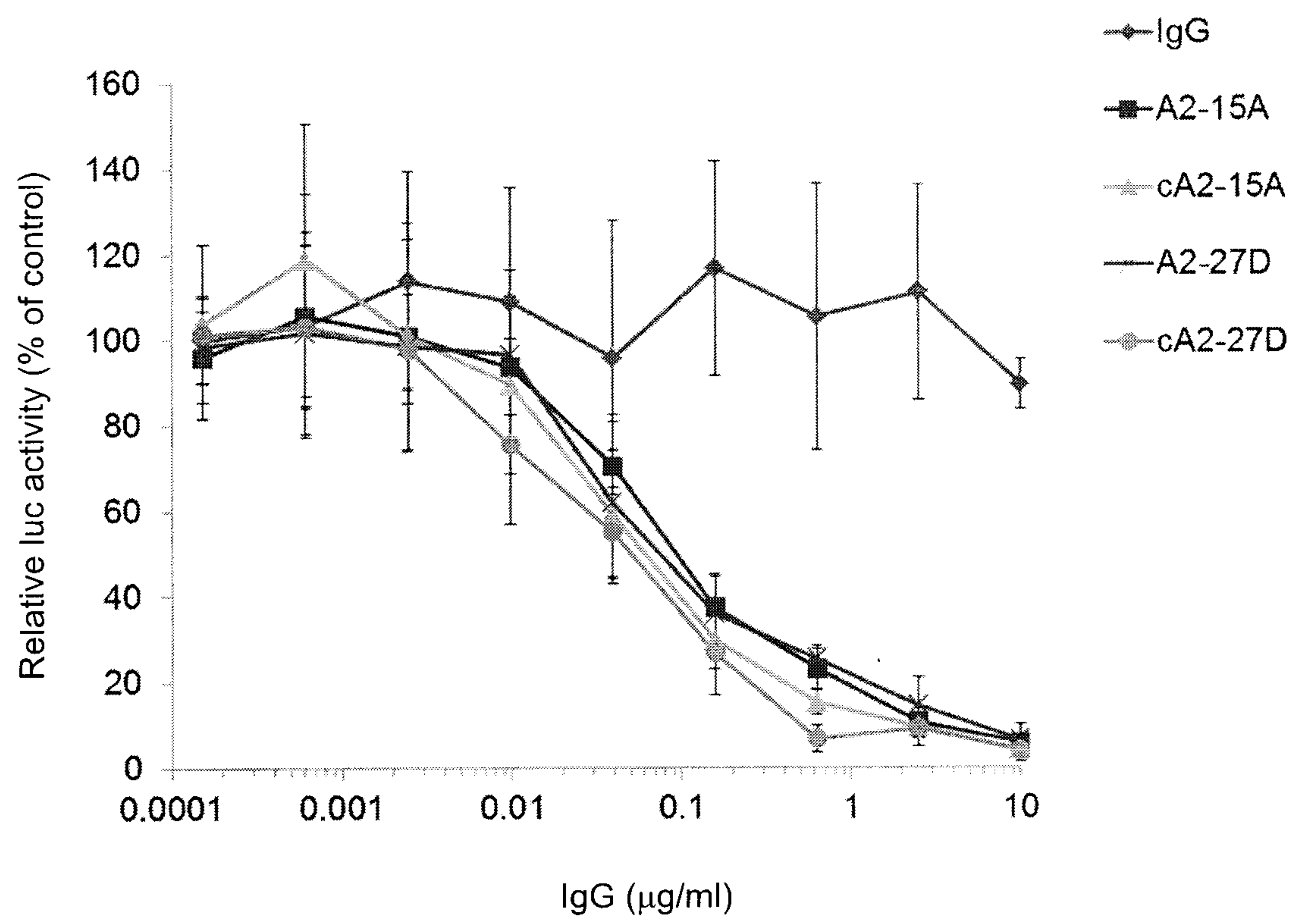


FIG. 10

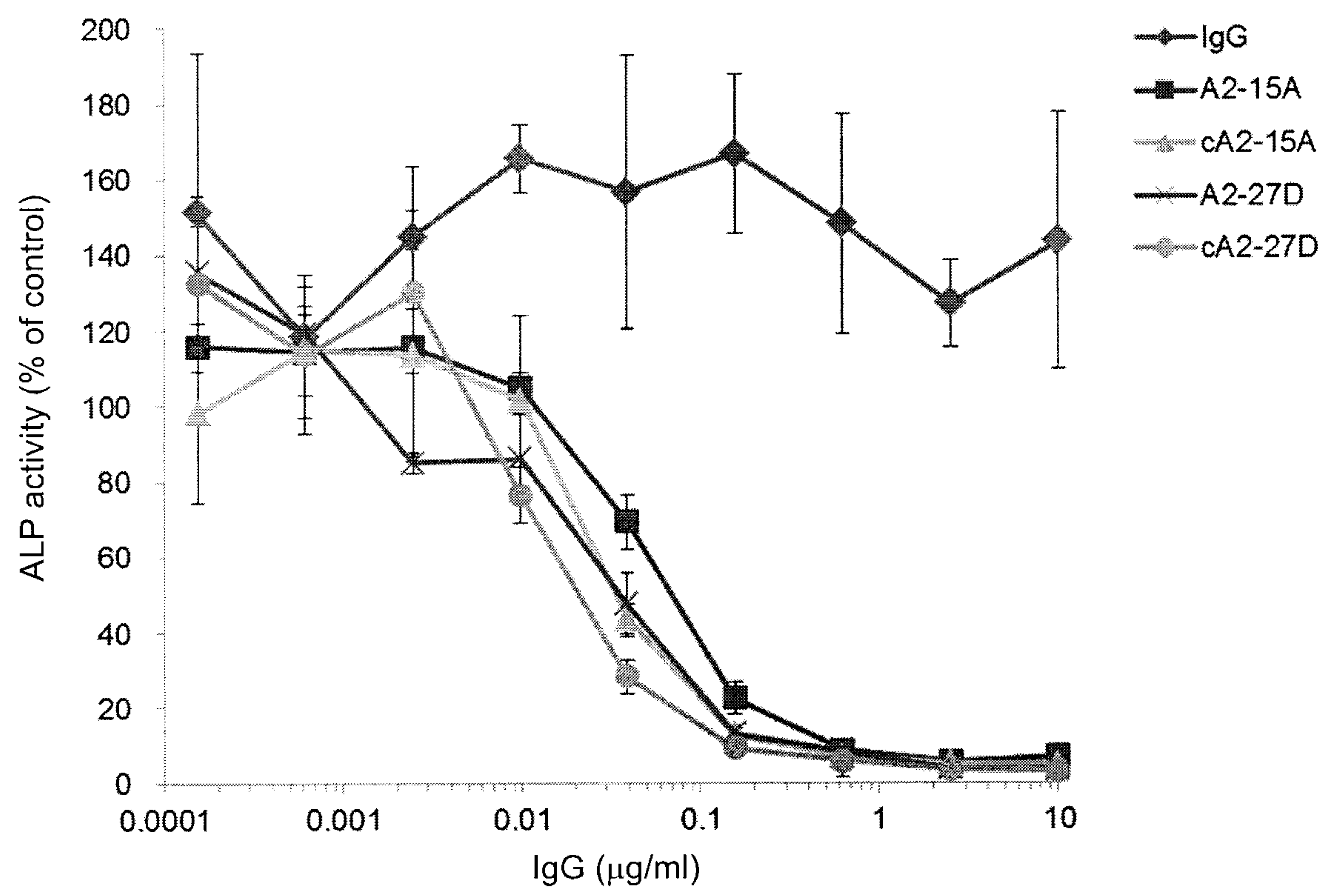


FIG. 11

SEQ ID NO:59: Amino acid sequence of A2-15A CDRH1
GFTFSHYMA

SEQ ID NO:60: Amino acid sequence of A2-15A CDRH2
SITNSGGSINYRDSVKG

SEQ ID NO: 61: Amino acid sequence of A2-15A CDRH3
EGGENYGGYPPFAY

SEQ ID NO:62: Amino acid sequence of A2-15A CDRL1
RANQGVSLSRYNLMH

SEQ ID NO:63: Amino acid sequence of A2-15A CDRL2
RSSNLAS

SEQ ID NO:64: Amino acid sequence of A2-15A CDRL3
QQSRESPFT

SEQ ID NO:71: Amino acid sequence of humanized hA2-15A-L6 CDRL2
RSSNLAQ

FIG. 12

SEQ ID NO:65: Amino acid sequence of A2-27D CDRH1
GSTFSNYGMK

SEQ ID NO:66: Amino acid sequence of A2-27D CDRH2
SISRSSTYIYYADTVKG

SEQ ID NO:67: Amino acid sequence of A2-27D CDRH3
AISTPFYWYFDF

SEQ ID NO:68: Amino acid sequence of A2-27D CDRL1
LASSSVSYMT

SEQ ID NO:69: Amino acid sequence of A2-27D CDRL2
GTSNLAS

SEQ ID NO:70: Amino acid sequence of A2-27D CDRL3
LHLTSYPPYT

FIG. 13

SEQ ID NO:72: Amino acid sequence of A2-11E CDRH1
GFTFSNYYMY

SEQ ID NO:73: Amino acid sequence of A2-11E CDRH2
SINTDGGSTIYPDSVKG

SEQ ID NO:74: Amino acid sequence of A2-11E CDRH3
STPNIPLAY

SEQ ID NO:75: Amino acid sequence of A2-11E CDRL1
KASQNIYKYLN

SEQ ID NO:76: Amino acid sequence of A2-11E CDRL2
YSNSLQT

SEQ ID NO:77: Amino acid sequence of A2-11E CDRL3
FQYSSGPT

FIG. 14

SEQ ID NO:78: Amino acid sequence of A2-25C CDRH1
GFTFSYYAMS

SEQ ID NO:79: Amino acid sequence of A2-25C CDRH2
SISRGGDNTYYRDTVKG

SEQ ID NO:80: Amino acid sequence of A2-25C CDRH3
LNYNIFYDY

SEQ ID NO:81: Amino acid sequence of A2-25C CDRL1
QASQDIGNWLS

SEQ ID NO:82: Amino acid sequence of A2-25C CDRL2
GATSLAD

SEQ ID NO:83: Amino acid sequence of A2-25C CDRL3
LQAYSAPFT

FIG. 15

SEQ ID NO:27: Nucleotide sequence of humanized hA2-15A-H1
ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCTTCACCTTCAG
CCACTACTACATGGCCTGGGTGCGCCAGGCCCCCTGGCAAAGGACTGGAATGGGTGGCCAGCATCACCAACAGC
GGCGGCAGCATCAACTACCGGGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCC
TGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTATTGTGCCAGAGAGGGCGGCGAGAA
CTACGGCGGCTATCCCCCTTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACCGTCAGCTCAGCCTCCACCAAG
GGCCCAAGCGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGG
TCAAGGACTACTTCCCCGAACCCGTGACCGTGAGCTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTT
CCCCGCTGTCCTGCAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGC
ACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAAT
CTTGTGACAAAACCTCACACATGCCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTCTT
CCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGC
CACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCCC
GGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGG
CAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAA
GGCCAGCCCCCGGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCC
TGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCCGAGAA
CAACTACAAGACCACCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGAC
AAGAGCAGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCC
AGAAGAGCCTCTCCCTGTCTCCCGGCAAA

Signal sequence (1-57), Variable region (58-426), Constant region (427-1416)

SEQ ID NO:28: Amino acid sequence of humanized hA2-15A-H1
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYMAWVRQAPGKGLEWVASITNS
GGSINYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREGGENYGGYPPFAYWGQGLTVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVD
KSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

Signal sequence (1-19), Variable region (20-142), Constant region (143-472)

FIG. 16

SEQ ID NO:29: Nucleotide sequence of humanized hA2-15A-H4
ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCTTCACCTTCAG
CCACTACTACATGGCCTGGGTGCGCCAGGCCCCCTGGCAAAGGACTGGAATGGGTGGCCAGCATCACCAACAGC
GGCGGCAGCATCAACTACCGGGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACGCCAAGAGCACCC
TGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCACCTACTACTGTACAAGAGAGGGCGGCGAGAA
CTACGGCGGCTACCCTCCTTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACCGTCAGCTCAGCCTCCACCAAG
GGCCCAAGCGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGG
TCAAGGACTACTTCCCCGAACCCGTGACCGTGAGCTGGAATCAGGCGCCCTGACCAGCGGCGTGCACACCTT
CCCCGCTGTCCTGCAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGC
ACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAAT
CTTGTGACAAAACCTCACACATGCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTCTT
CCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGC
CACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCCC
GGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGG
CAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAA
GGCCAGCCCCCGGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCC
TGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAA
CAACTACAAGACCACCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGAC
AAGAGCAGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCC
AGAAGAGCCTCTCCCTGTCTCCCGGCAAA
Signal sequence (1-57), Variable region (58-426), Constant region (427-1416)

SEQ ID NO:30: Amino acid sequence of humanized hA2-15A-H4
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYMAWVRQAPGKGLEWVASITNS
GGSINYRDSVKGRFTISRDNASTLYLQMNSLRAEDTATYYCTREGGENYGGYPPFAYWGQGLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
TQTYICNVNHKPSNTKVDKRVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVD
KSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
Signal sequence (1-19), Variable region (20-142), Constant region (143-472)

FIG. 17

SEQ ID NO:31: Nucleotide sequence of DNA fragment comprising sequences encoding humanized hA2-15A-L1
ccagcctccggactctagagccaccATGGTGCTGCAGACCCAGGTGTTTCATCAGCCTGCTGCTGTGGATCAGC
GGCGCCTACGGCGACATCGTGATGACCCAGAGCCCTGACAGCCTGGCCGTGTCTCTGGGAGAGAGGGCCACCA
TCAACTGCAGAGCCAACCAGGGCGTGTCCCTGAGCCGGTACAACCTGATGCACTGGTATCAGCAGAAGCCCGG
CCAGCCCCCAAGCTGCTGATCTACAGAAGCTCCAACCTGGCCAGCGGCGTGCCCGATAGATTTTCTGGCAGC
GGCTCCGGCACCGACTTCACCCTGACAATCAGCTCCCTGCAGGCCGAGGACGTGGCCGTGTACTACTGCCAGC
AGAGCAGAGAGAGCCCTTCACCTTTGGCCAGGGCACCAAGGTGGAAATCAAGCGGGCTGTGGCCGCTCCCTC
CGTGTTTCATCTTTCCACCCAGCGACGAGCAGCTGAAGTCTGGCACAGCCAGCGTCGTGTGCCTGCTGAACAAC
TTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAATGCCCTGCAGAGCGGCAACTCCCAGGAAAGCG
TGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCCTGACCCTGAGCAAGGCCGACTACGA
GAAGCACAAGGTGTACGCCTGCGAAGTGACCCACCAGGGCCTGTCTAGCCCCGTGACCAAGAGCTTCAACCGG
GGCGAGTGTtgagtttaaacgggggaggctaact
Signal sequence (26-85), Variable region (86-424), Constant region (425-739)

SEQ ID NO:32: Amino acid sequence of humanized hA2-15A-L1
MVLQTQVFISLLWISGAYGDIVMTQSPDSLAVSLGERATINCRANQGVSLSRYNLMHWYQQKPGQPPKLLIY
RSSNLASGVPDRFSGSGSDFTLTISSLQAEDVAVYYCQQSRESPFTFGQGTKVEIKRAVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC
Signal sequence (1-20), Variable region (21-133), Constant region (134-238)

FIG. 18

SEQ ID NO:33: Nucleotide sequence of DNA fragment comprising sequences encoding humanized hA2-15A-L4
ccagcctccggactctagagccaccATGGTGCTGCAGACCCAGGTGTTTCATCAGCCTGCTGCTGTGGATCAGC
GGCGCCTACGGCGATATCGTGCTGACCCAGAGCCCTGACAGCCTGGCCGTGTCTCTGGGAGAGAGGGCCACCA
TCAACTGCAGAGCCAACCAGGGCGTGCCCTGAGCCGGTACAACCTGATGCACTGGTATCAGCAGAAGCCCGG
CCAGAAGCCCAAGCTGCTGATCTACCGGTCCAGCAACCTGGCCTCTGGCATCCCCGCCAGATTTTCTGGCAGC
GGCTCCGGCACCGACTTCACCCTGACAATCAGCAGCGTGCAGGCCGACGACATTGCCGTGTACTACTGCCAGC
AGAGCAGAGAGAGCCCCTTCACCTTTGGCCAGGGCACCAAGCTGGAAGTGAAGAGAGCCGTGGCCGCTCCCTC
CGTGTTTCATCTTCCCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCAGCGTCGTGTGCCTGCTGAACAAC
TTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAAATGCCCTGCAGAGCGGCAACAGCCAGGAAAGCG
TGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCCTGACCCTGAGCAAGGCCGACTACGA
GAAGCACAAAGGTGTACGCCTGCGAAGTGACCCACCAGGGCCTGTCTAGCCCCGTGACCAAGAGCTTCAACCGG
GGCGAGTGTTgagtttaaacgggggaggctaact
Signal sequence (26-85), Variable region (86-424), Constant region (425-739)

SEQ ID NO:34: Amino acid sequence of humanized hA2-15A-L4
MVLQTQVFISLLLWISGAYGDIVLTQSPDSLAVSLGERATINCRANQGVSLSRYNLMHWYQQKPGQKPKLLIY
RSSNLASGIPARFSGSGSGTDFTLTISSVQADDIAVYYCQQSRESPFTFGQGTKLELKRAVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC
Signal sequence (1-20), Variable region (21-133), Constant region (134-238)

FIG. 19

SEQ ID NO:35: Nucleotide sequence of DNA fragment comprising sequences encoding humanized hA2-15A-L6

ccagcctccggactctagagccaccATGGTGCTGCAGACCCAGGTGTTTCATCAGCCTGCTGCTGTGGATCAGC
GGCGCCTACGGCGATATCGTGCTGACCCAGAGCCCTGACAGCCTGGCCGTGTCTCTGGGAGAGAGGGCCACCA
TCAACTGCAGAGCCAACCAGGGCGTGTCCCTGAGCCGGTACAACCTGATGCACTGGTATCAGCAGAAGCCCGG
CCAGAAGCCCAAGCTGCTGATCTACCGGTCCAGCAACCTGGCCCAGGGCATCCCTGCCAGATTTTCTGGCAGC
GGCTCCGGCACC GACTTCACCCTGACAATCAGCAGCGTG CAGGCCGACGACATTGCCGTGTACTACTGCCAGC
AGAGCAGAGAGAGCCCTTCACCTTTGGCCAGGGCACCAAGCTGGAAGTGAAGAGAGCCGTGGCCGCTCCCTC
CGTGTTTCATCTTCCCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCAGCGTCGTGTGCCTGCTGAACAAC
TTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAATGCCCTGCAGAGCGGCAACAGCCAGGAAAGCG
TGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCCTGACCCTGAGCAAGGCCGACTACGA
GAAGCACAAGGTGTACGCCTGCGAAGTGACCCACCAGGGCCTGTCTAGCCCCGTGACCAAGAGCTTCAACCGG
GGCGAGTGTtgagtttaaacgggggaggctaact

Signal sequence (26-85), Variable region (86-424), Constant region (425-739)

SEQ ID NO:36: Amino acid sequence of humanized hA2-15A-L6

MVLQTQVFISLLLWISGAYGDIVLTQSPDSLAVSLGERATINCRANQGVSLSRYNLMHWYQQKPGQKPKLLIY
RSSNLAQGIPARFSGSGSGTDFTLTISVQADDIAVYYCQQSRESPFTFGQGTKLELKRAVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC

Signal sequence (1-20), Variable region (21-133), Constant region (134-238)

FIG. 20

SEQ ID NO:37: Nucleotide sequence of DNA fragment comprising sequences encoding humanized hA2-15A-L7
ccagcctccggactctagagccaccATGGTGCTGCAGACCCAGGTGTTTCATCAGCCTGCTGCTGTGGATCAGC
GGCGCCTACGGCGATATCGTGCTGACCCAGAGCCCTGACAGCCTGGCCGTGTCTCTGGGAGAGAGGGCCACCA
TCAACTGCAGAGCCAACCAGGGCGTGTCCCTGAGCCGGTACAACCTGATGCACTGGTATCAGCAGAAGCCCGG
CCAGAAGCCCAAGCTGGCCATCTACAGAAGCAGCAACCTGGCCAGCGGCATCCCCGCCAGATTTTCTGGCAGC
GGCTCCGGCACCGACTTCACCCTGACAATCAGCAGCGTGAGGCCGACGACATTGCCGTGTACTACTGCCAGC
AGAGCAGAGAGAGCCCCTTCACCTTTGGCCAGGGCACCAAGCTGGAAGTGAAGAGAGCCGTGGCCGCTCCCTC
CGTGTTTCATCTTCCCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCAGCGTCGTGTGCCTGCTGAACAAC
TTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAATGCCCTGCAGAGCGGCAACAGCCAGGAAAGCG
TGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCCTGACCCTGAGCAAGGCCGACTACGA
GAAGCACAAGGTGTACGCCTGCCAAGTGACCCACCAGGGCCTGTCTAGCCCCGTGACCAAGAGCTTCAACCGG
GGCGAGTGTtgagtttaaacgggggaggctaact
Signal sequence (26-85), Variable region (86-424), Constant region (425-739)

SEQ ID NO:38: Amino acid sequence of humanized hA2-15A-L7
MVLQTQVFISLLLWISGAYGDIVLTQSPDSLAVSLGERATINCRANQGVSLSRYNLMHWYQQKPGQKPKLAIY
RSSNLASGIPARFSGSGSGTDFTLTISVQADDIAVYYCQQSRESPFTFGQGTKLELKRAVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC
Signal sequence (1-20), Variable region (21-133), Constant region (134-238)

FIG. 21

SEQ ID NO:39: Nucleotide sequence of humanized hA2-27D-H1

ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCAGCACCTTCAG
CAACTACGGCATGAAATGGGTGCGCCAGGCCCCCTGGCAAGGGACTGGAATGGGTGTCCAGCATCAGCAGAAGC
AGCACCTACATCTACTACGCCGACACCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCC
TGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTATTGTGCCAGAGCCATCAGCACCCC
CTTCTACTGGTACTTCGACTTCTGGGGCCAGGGCACCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCA
AGCGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCCGTGACCGTGAGCTGGAATCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCCGC
TGTCTGCAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG
ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCCAAATCTTGTG
ACAAAACCTCACACATGCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCTCTTCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA
GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGG
AGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACCCTGCACCAGGACTGGCTGAATGGCAAGGA
GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAGGCCAG
CCCCGGGAACCACAGGTGTACACCCTGCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTACGCTGACCT
GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTA
CAAGACCACCCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCCTGGACAAGAGC
AGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCCAGAAGA
GCCTCTCCCTGTCTCCCGGCAAA

Signal sequence (1-57), Variable region (58-420), Constant region (421-1410)

SEQ ID NO:40: Amino acid sequence of humanized hA2-27D-H1

MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGSTFSNYGMKWVRQAPGKGLEWVSSISRS
STYIYYADTVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARAISTPFYWFDFWGQGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Signal sequence (1-19), Variable region (20-140), Constant region (141-470)

FIG. 22

SEQ ID NO:41: Nucleotide sequence of humanized hA2-27D-H2
ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCAGCACCTTCAG
CAACTACGGCATGAAGTGGATCCGGCAGGCCCCCTGGCAAGGGCCTGGAATGGGTGTCCAGCATCAGCAGAAGC
AGCACCTACATCTACTACGCCGACACCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCC
TGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTATTGTGCCGCCGCTATCAGCACCCC
CTTCTACTGGTACTTCGACTTCTGGGGCCAGGGCACCCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCA
AGCGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCCGTGACCGTGAGCTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCCGC
TGCCTGCAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG
ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCCAAATCTTGTG
ACAAAACCTCACACATGCCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTCTTCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA
GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGG
AGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAGGA
GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGCCAG
CCCCGGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCT
GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAATA
CAAGACCACCCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGC
AGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCCAGAAGA
GCCTCTCCCTGTCTCCCGGCAAA
Signal sequence (1-57), Variable region (58-420), Constant region (421-1410)

SEQ ID NO:42: Amino acid sequence of humanized hA2-27D-H2
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGSTFSNYGMKWIRQAPGKGLEWVSSISRS
STYIYYADTVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAA AISTPFYWFDFWGQGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Signal sequence (1-19), Variable region (20-140), Constant region (141-470)

FIG. 23

SEQ ID NO:43: Nucleotide sequence of humanized hA2-27D-H3
ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCAGCACCTTCAG
CAACTACGGCATGAAGTGGATCCGGCAGGCCCCCTGGCAAGGGCCTGGAATGGGTGGCCAGCATCAGCAGAAGC
AGCACCTACATCTACTACGCCGACACCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCC
TGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTATTGTGCCCGCGCTATCAGCACCCC
CTTCTACTGGTACTTCGACTTCTGGGGCCAGGGCACCCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCA
AGCGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCCGTGACCGTGAGCTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCCGC
TGTCTGCAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG
ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTG
ACAAAACCTCACACATGCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTCTTCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA
GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGG
AGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGA
GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGCCAG
CCCCGGGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTACGCCTGACCT
GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTA
CAAGACCACCCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGC
AGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCCAGAAGA
GCCTCTCCCTGTCTCCCGGCAAA
Signal sequence (1-57), Variable region (58-420), Constant region (421-1410)

SEQ ID NO:44: Amino acid sequence of humanized hA2-27D-H3
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGSTFSNYGMKWIRQAPGKGLEWVASISRS
STYIYYADTVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAAAISTPFYWFDFWGQGLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Signal sequence (1-19), Variable region (20-140), Constant region (141-470)

FIG. 24

SEQ ID NO:45: Nucleotide sequence of humanized hA2-27D-H4

ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCAGCACCTTCAG
CAACTACGGCATGAAGTGGATCCGGCAGGCCCCCTGGCAAGGGCCTGGAATGGGTGGCCAGCATCAGCAGAAGC
AGCACCTACATCTACTACGCCGACACCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCC
TGTAACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCCTGTACTATTGTGCCGCCGCTATCAGCACCCC
CTTCTACTGGTACTTCGACTTCTGGGGCCCTGGCACCCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCA
AGCGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCCGTGACCGTGAGCTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCCGC
TGTCTGCAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG
ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCCAAATCTTGTG
ACAAAACCTCACACATGCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTCTTCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA
GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGG
AGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAGGA
GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGCCAG
CCCCGGAACACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCT
GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTA
CAAGACCACCCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCCTGGACAAGAGC
AGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCCAGAAGA
GCCTCTCCCTGTCTCCCGGCAAA

Signal sequence (1-57), Variable region (58-420), Constant region (421-1410)

SEQ ID NO:46: Amino acid sequence of humanized hA2-27D-H4

MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGSTFSNYGMKWIRQAPGKGLEWVASISRS
STYIYYADTVKGRFTISRDN SKNTLYLQMNSLRAEDTALYYCAA AISTPFYWYFD FWGPGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKS
RWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

Signal sequence (1-19), Variable region (20-140), Constant region (141-470)

FIG. 25

SEQ ID NO:47: Nucleotide sequence of humanized hA2-27D-H5
ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCAGCACCTTCAG
CAACTACGGCATGAAGTGGATCCGGCAGGCCCCCTGGCAAGGGCCTGGAATGGGTGGCCAGCATCAGCAGAAGC
AGCACCTACATCTACTACGCCGACACCGTGAAGGGCCGGTTCACCATCTCCCGGGACAACGCCAAGAACACCC
TGTACCTGCAGATGAACAGCCTGCGGAGCGAGGACACCGCCCTGTACTATTGTGCCGCCGCTATCAGCACCCC
CTTCTACTGGTACTTCGACTTCTGGGGCCCTGGCACCCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCA
AGCGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCCGTGACCGTGAGCTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCCGC
TGTCTGCAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG
ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCCAAATCTTGTG
ACAAAACCTCACACATGCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTCTTCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA
GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGG
AGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAGGA
GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGCCAG
CCCCGGAACACAGGTGTACACCCTGCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTACGCTGACCT
GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTA
CAAGACCACCCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGC
AGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCCAGAAGA
GCCTCTCCCTGTCTCCCGGCAAA
Signal sequence (1-57), Variable region (58-420), Constant region (421-1410)

SEQ ID NO:48: Amino acid sequence of humanized hA2-27D-H5
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGSTFSNYGMKWIRQAPGKGLEWVASISRS
STYIYYADTVKGRFTISRDNKNTLYLQMNSLRSEDTALYYCAAISTPFYWFDFWGPGLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Signal sequence (1-19), Variable region (20-140), Constant region (141-470)

FIG. 26

SEQ ID NO:49: Nucleotide sequence of DNA fragment comprising sequences encoding humanized hA2-27D-L1
ccagcctccggactctagagccaccATGGTGCTGCAGACCCAGGTGTTTCATCAGCCTGCTGCTGTGGATCAGC
GGCGCCTACGGCGAGATCGTGCTGACACAGAGCCCTGGCACCCCTGAGCCTGTCTCCAGGCGAAAGAGCCACCC
TGTCTGTCTGGCCAGCAGCAGCGTGTCTACATGACCTGGTATCAGCAGAAGCCCGGCCAGGCCCCCAGACT
GCTGATCTACGGCACAAGCAATCTGGCCTCCGGCATCCCCGACAGATTTTCCGGCTCTGGCAGCGGCACCGAC
TTCACCCTGACCATCAGCAGACTGGAACCCGAGGACTTCGCCGTGTACTACTGCCTGCACCTGACCAGCTACC
CCCCCTACACATTTGGCCAGGGCACCAAGGTGGAATCAAGCGGGCTGTGGCCGCTCCCTCCGTGTTTCATCTT
TCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAGCTAGCGTCGTGTGCCTGCTGAACAACTTCTACCCCCGC
GAGGCCAAGGTGCAGTGGAAGGTGGACAATGCCCTGCAGAGCGGCAACTCCCAGGAAAGCGTGACCGAGCAGG
ACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCCTGACACTGAGCAAGGCCGACTACGAGAAGCACAAGGT
GTACGCCTGCCAAGTGACCCACCAGGGCCTGTCTAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGtga
gtttaaacgggggaggctaact
Signal sequence (26-85), Variable region (86-412), Constant region (413-727)

SEQ ID NO:50: Amino acid sequence of humanized hA2-27D-L1
MVLQTQVFISLLWISGAYGEIVLTQSPGTLSPGERATLSCLASSSVSYMTWYQQKPGQAPRLLIYGTSNL
ASGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCLHLTSYPPTYTFGQGTKVEIKRAVAAPSVFIFPPSDEQLK
SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC
Signal sequence (1-20), Variable region (21-129), Constant region (130-234)

FIG. 27

SEQ ID NO:51: Nucleotide sequence of DNA fragment comprising sequences encoding humanized hA2-27D-L2

ccagcctccggactctagagccaccATGGTGCTGCAGACCCAGGTGTTTCATCAGCCTGCTGCTGTGGATCAGC
GGCGCCTACGGCGAGATCGTGCTGACACAGAGCCCTGGCACCCCTGAGCCTGTCTCCAGGCGAAAGAGCCACCC
TGTCCTGTCTGGCCAGCAGCAGCGTGTCTTACATGACCTGGTATCAGCAGAAGCCCGGCCAGAGCCCCAGACT
GTGGATCTACGGCACCAGCAATCTGGCCTCCGGCGTGCCCGATAGATTTTCCGGCTCTGGCAGCGGCACCGAC
TTCACCCTGACCATCAGCAGACTGGAACCCGAGGACTTCGCCGTGTACTACTGCCTGCACCTGACCAGCTACC
CCCCCTACACATTTGGCCAGGGCACCAAGGTGGAAATCAAGCGGGCTGTGGCCGCTCCCTCCGTGTTTCATCTT
TCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAGCTAGCGTCGTGTGCCTGCTGAACAACTTCTACCCCCGC
GAGGCCAAGGTGCAGTGGAAGGTGGACAATGCCCTGCAGAGCGGCAACTCCCAGGAAAGCGTGACCGAGCAGG
ACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCCTGACACTGAGCAAGGCCGACTACGAGAAGCACAAAGGT
GTACGCCTGCGAAGTGACCCACCAGGGCCTGTCTAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGTtga
gtttaaacgggggaggctaact

Signal sequence (26-85), Variable region (86-412), Constant region (413-727)

SEQ ID NO:52: Amino acid sequence of humanized hA2-27D-L2

MVLQTQVFISLLLWISGAYGEIVLTQSPGTLSLSPGERATLSCLASSSVSYMTWYQQKPGQSPRLWIYGTSNL
ASGVPDRFSGSGSGTDFTLTISRLEPEDFAVYYCLHLTSYPPYTFGQGTKVEIKRAVAAPSVFIFPPSDEQLK
SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Signal sequence (1-20), Variable region (21-129), Constant region (130-234)

FIG. 28

SEQ ID NO:53: Nucleotide sequence of DNA fragment comprising sequences encoding humanized hA2-27D-L3
ccagcctccggactctagagccaccATGGTGCTGCAGACCCAGGTGTTTCATCAGCCTGCTGCTGTGGATCAGC
GGCGCCTACGGCGAGATCGTGCTGACACAGAGCCCTGGCACCCCTGAGCCTGTCTCCAGGCGAAAGAGCCACCC
TGTCTGTCTGGCCAGCAGCAGCGTGTCTTACATGACCTGGTATCAGCAGAAGCCAGGCGCCAGCCCCAGACT
GTGGATCTACGGCACAAGCAACCTGGCCAGCGCGTGCCGATAGATTTTCTGGCAGCGGCTCCGGCACCGAC
TACACCCTGACAATCAGCAGACTGGAACCCGAGGACTTCGCCACCTACTACTGCCTGCACCTGACCAGCTACC
CCCCCTACACATTTGGAGCCGGCACCAAGGTGGAAATCAAGCGGGCTGTGGCCGCTCCCTCCGTGTTTCATCTT
TCCACCCAGCGACGAGCAGCTGAAGTCTGGCACAGCCAGCGTCGTGTGCCTGCTGAACAACTTCTACCCCCGC
GAGGCCAAGGTGCAGTGGAAGGTGGACAATGCCCTGCAGAGCGGCAACTCCCAGGAAAGCGTGACCGAGCAGG
ACAGCAAGGACTCCACCTACAGCCTGAGCAGCACACTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGT
GTACGCCTGCGAAGTGACCCACCAGGGCCTGTCTAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGTga
gtttaaacgggggaggctaact
Signal sequence (26-85), Variable region (86-412), Constant region (413-727)

SEQ ID NO:54: Amino acid sequence of humanized hA2-27D-L3
MVLQTQVFISLLWISGAYGEIVLTQSPGTLSPGERATLSCLASSSVSYMTWYQQKPGASPRLLWIYGTSNL
ASGVPDRFSGSGSDYTLTISRLEPEDFATYYCLHLTSYPPYTFGAGTKVEIKRAVAAPSVFIFPPSDEQLK
SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC
Signal sequence (1-20), Variable region (21-129), Constant region (130-234)

FIG. 29

SEQ ID NO:55: Nucleotide sequence of DNA fragment comprising sequences encoding humanized hA2-27D-L4
ccagcctccggactctagagccaccATGGTGCTGCAGACCCAGGTGTTTCATCAGCCTGCTGCTGTGGATCAGC
GGCGCCTACGGCGAGATCGTGCTGACACAGAGCCCTGGCACCATGTCTGCCAGCCCTGGCGAGAGAGTGACCC
TGAGCTGTCTGGCCAGCTCCAGCGTGCCTACATGACCTGGTATCAGCAGAAGCCAGGCGCCAGCCCCAGACT
GTGGATCTACGGCACAAGCAACCTGGCCAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCCGGCACCGAC
TACACCCTGACCATCAGCCGGATGGAACCCGAGGACTTCGCCACCTACTACTGCCTGCACCTGACCAGCTACC
CCCCCTACACATTTGGAGCCGGCACCAAGCTGGAAGTGAAGAGAGCCGTGGCTGCCCCCTCCGTGTTTCATCTT
CCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCAGCGTCGTGTGCCTGCTGAACAACCTTCTACCCCCGC
GAGGCCAAGGTGCAGTGGAAGGTGGACAATGCCCTGCAGAGCGGCAACAGCCAGGAAAGCGTGACCGAGCAGG
ACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCCTGACACTGAGCAAGGCCGACTACGAGAAGCACAAAGT
GTACGCCTGCGAAGTGACCCACCAGGGCCTGTCTAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGtga
gtttaaacgggggaggctaact
Signal sequence (26-85), Variable region (86-412), Constant region (413-727)

SEQ ID NO:56: Amino acid sequence of humanized hA2-27D-L4
MVLQTQVFISLLLWISGAYGEIVLTQSPGTMSASPGERVTLSSCLASSSVSYMTWYQQKPGASPRLWIYGTSNL
ASGVDPDRFSGSGSGTDYTLTISRMEPEDFATYYCLHLTSYPPYTFGAGTKLELKRAVAAPSVFIFPPSDEQLK
SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC
Signal sequence (1-20), Variable region (21-129), Constant region (130-234)

FIG. 30

SEQ ID NO:57: Nucleotide sequence of DNA fragment comprising sequences encoding humanized hA2-27D-L5

ccagcctccggactctagagccaccATGGTGCTGCAGACCCAGGTGTTTCATCAGCCTGCTGCTGTGGATCAGC
GGCGCCTACGGCGAGATCGTGCTGACACAGAGCCCTGGCACCATGTCTGCCAGCCCTGGCGAGAGAGTGACCC
TGAATTGTCTGGCCAGCAGCAGCGTGTCTTACATGACCTGGTATCAGCAGAAGTCCGGCGCCAGCCCCAACT
GTGGATCTACGGCACCAGCAACCTGGCCAGCGGCGTGCCCAATAGATTTTCCGGCAGCGGCTCCGGCACCTCC
TACACCCTGACCATCAGCCGGATGGAACCCGAGGACTTCGCCACCTACTACTGCCTGCACCTGACCAGCTACC
CCCCCTACACATTTGGAGCCGGCACCAAGCTGGAAGTGAAGAGAGCCGTGGCCGCTCCCTCCGTGTTTCATCTT
CCCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCAGCGTCGTGTGCCTGCTGAACAACTTCTACCCCCGC
GAGGCCAAGGTGCAGTGGAAGGTGGACAATGCCCTGCAGAGCGGCAACAGCCAGGAAAGCGTGACCGAGCAGG
ACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCCTGACACTGAGCAAGGCCGACTACGAGAAGCACAAGGT
GTACGCCTGCGAAGTGACCCACCAGGGCCTGTCTAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGtga
gtttaaacggggaggctaact

Signal sequence (26-85), Variable region (86-412), Constant region (413-727)

SEQ ID NO:58: Amino acid sequence of humanized hA2-27D-L5

MVLQTQVFISLLLWISGAYGEIVLTQSPGTMSASPGERVTLNCLASSSVSYMTWYQQKSGASPKLWIYGTSNL
ASGVPNRFSGSGSGTSYTLTISRMEPEDFATYYCLHLTSYPPYTFGAGTKLELKRAVAAPSVFIFPPSDEQLK
SGTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Signal sequence (1-20), Variable region (21-129), Constant region (130-234)

FIG. 31

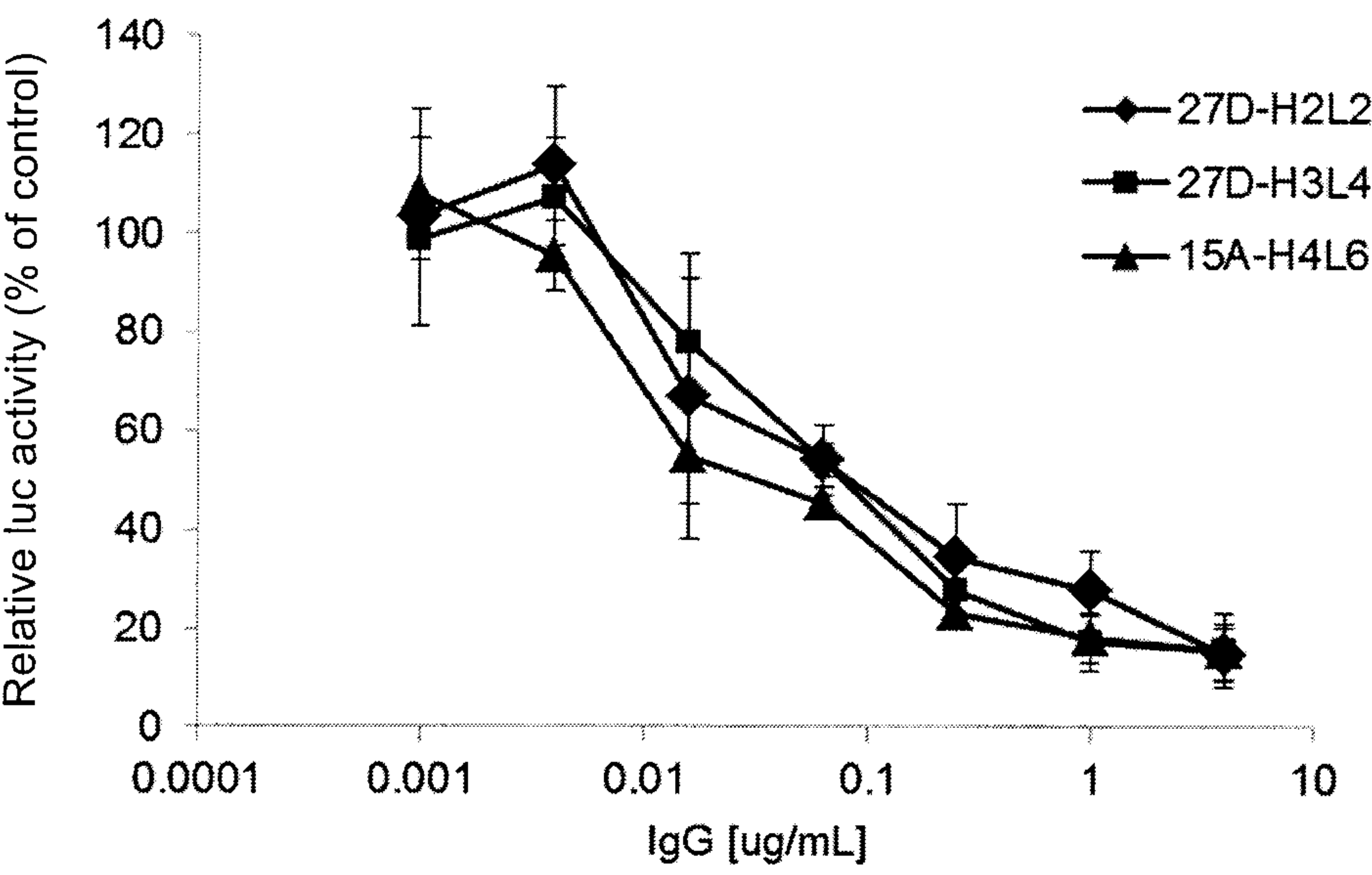


FIG. 32

SEQ ID NO:104: Nucleotide sequence of humanized hA2-15A-H4 IgG2 type
ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCTTCACCTTCAG
CCACTACTACATGGCCTGGGTGCGCCAGGCCCCCTGGCAAAGGACTGGAATGGGTGGCCAGCATCACCAACAGC
GGCGGCAGCATCAACTACCGGGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACGCCAAGAGCACCC
TGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCACCTACTACTGTACAAGAGAGGGCGGCGAGAA
CTACGGCGGCTACCTCCTTTTGCCTACTGGGGCCAGGGCACCTCGTGACCGTCAGCTCAGCCAGCACCAAG
GGCCCTTCCGTGTTCCCTCTGGCCCCCTTGTAGCCGTTCCACCAGCGAGTCCACCGCCGCCCTTGGCTGTCTGG
TGAAGGACTACTTCCCTGAGCCTGTGACCGTGAGCTGGAATCCGGAGCCCTTACCAGCGGCGTGACACACCTT
CCCTGCCGTGCTGCAGTCCAGCGGCCTTTACTCCCTGAGCTCCGTGGTGACCGTGCCTAGCTCCAACCTCGGC
ACCCAAACCTACACCTGTAACGTGGACCACAAGCCTAGCAACACCAAGGTGGACAAGACCGTGGAGCGTAAGT
GTTGTGTGGAGTGTCTCCTTGTCTGCCCCCTCCTGTGGCCGGACCTTCCGTGTTCCTTTTCCCTCCTAAGCC
TAAGGACACCCTGATGATCAGCCGTACCCCTGAGGTGACCTGTGTGGTGGTGGACGTGTCCCACGAGGACCCT
GAGGTGCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCTCGTGAGGAGCAAT
TCAACAGCACCTTCCGTGTGGTGTCCGTGCTTACCGTGGTGCACCAAGACTGGCTGAACGGCAAGGAGTACAA
GTGTAAGGTGAGCAACAAGGGACTTCCTGCCCCATCGAGAAGACCATCTCCAAGACCAAGGGCCAACCTCGT
GAGCCTCAAGTGTACACCCTTCCTCCTAGCCGTGAGGAGATGACCAAGAACCAAGTGTCCCTTACCTGTCTGG
TGAAGGGCTTCTACCCTAGCGACATCGCCGTGGAGTGGGAGTCCAACGGACAACCTGAGAACAACCTACAAGAC
CACCCCTCCTATGCTTGACAGCGACGGCTCCTTCTTCTGTACAGCAAGCTGACCGTGGACAAGTCCCGTTGG
CAACAAGGCAACGTGTTGAGCTGTTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAAAAGAGCCTTT
CCCTGAGCCCTGGAAAG

Signal sequence (1-57), Variable region (58-426), Constant region (427-1404)

SEQ ID NO:105: Amino acid sequence of humanized hA2-15A-H4 IgG2 type
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYMAWVRQAPGKGLEWVASITNS
GGSINYRDSVKGRFTISRDNASTLYLQMNSLRAEDTATYYCTREGGENYGGYPPFAYWGQGLVTVSSASTK
GPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFG
TQYTCNVDPKPSNTKVDKTVKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTFRVSVLTVVHQDNLNGKEYKCKVSNKGLPAPIEKTISKTKGQPR
EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPMLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Signal sequence (1-19), Variable region (20-142), Constant region (143-468)

FIG. 33

SEQ ID NO:106: Nucleotide sequence of humanized hA2-27D-H2-LALA

ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCAGCACCTTCAG
CAACTACGGCATGAAGTGGATCCGGCAGGCCCCCTGGCAAGGGCCCTGGAATGGGTGTCCAGCATCAGCAGAAGC
AGCACCTACATCTACTACGCCGACACCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCC
TGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTATTGTGCCGCCGCTATCAGCACCCC
CTTCTACTGGTACTTCGACTTCTGGGGCCAGGGCACCCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCA
AGCGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCCGTGACCGTGAGCTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCCGC
TGTCCCTGCAGTCCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG
ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTG
ACAAAACCTCACACATGCCCACCCTGCCCAGCACCTGAAGCCGCGGGGGACCCTCAGTCTTCCTCTTCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAA
GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGG
AGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAGGA
GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAGGCCAG
CCCCGGGAACACAGGTGTACACCCTGCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCT
GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAATA
CAAGACCACCCTCCCGTGTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCCTGGACAAGAGC
AGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCCAGAAGA
GCCTCTCCCTGTCTCCCGGCAAA

Signal sequence (1-57), Variable region (58-420), Constant region (421-1410)

SEQ ID NO:107: Amino sequence of humanized hA2-27D-H2-LALA

MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGSTFSNYGMKWIRQAPGKGLEWVSSISRS
STYIYYADTVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAA AISTPFYWFYDFWQQGLTVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKHTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKS
RWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

Signal sequence (1-19), Variable region (20-140), Constant region (141-470)

FIG. 34

SEQ ID NO:108: Nucleotide sequence of humanized hA2-27D-H3-LALA
ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCAGCACCTTCAG
CAACTACGGCATGAAGTGGATCCGGCAGGCCCCCTGGCAAGGGCCTGGAATGGGTGGCCAGCATCAGCAGAAGC
AGCACCTACATCTACTACGCCGACACCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCC
TGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTATTGTGCCGCCGCTATCAGCACCCC
CTTCTACTGGTACTTCGACTTCTGGGGCCAGGGCACCCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCCA
AGCGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAGG
ACTACTTCCCCGAACCCGTGACCGTGAGCTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCCGC
TGTCCCTGCAGTCCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG
ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTG
ACAAAACCTCACACATGCCCCACCCTGCCCAGCACCTGAAGCCGCGGGGGGACCCTCAGTCTTCCTCTTCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA
GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGG
AGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAGGA
GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGCCAG
CCCCGGGAACACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCT
GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAATA
CAAGACCACCCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCCTGGACAAGAGC
AGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCCAGAAGA
GCCTCTCCCTGTCTCCCGGCAAA

Signal sequence (1-57), Variable region (58-420), Constant region (421-1410)

SEQ ID NO:109: Amino sequence of humanized hA2-27D-H3-LALA
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGSTFSNYGMKWIRQAPGKLEWVASISRS
STYIYYADTVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAA AISTPFYWYFD F W G Q G T L V T V S S A S T K G P
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKHTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKS
RWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

Signal sequence (1-19), Variable region (20-140), Constant region (141-470)

FIG. 35

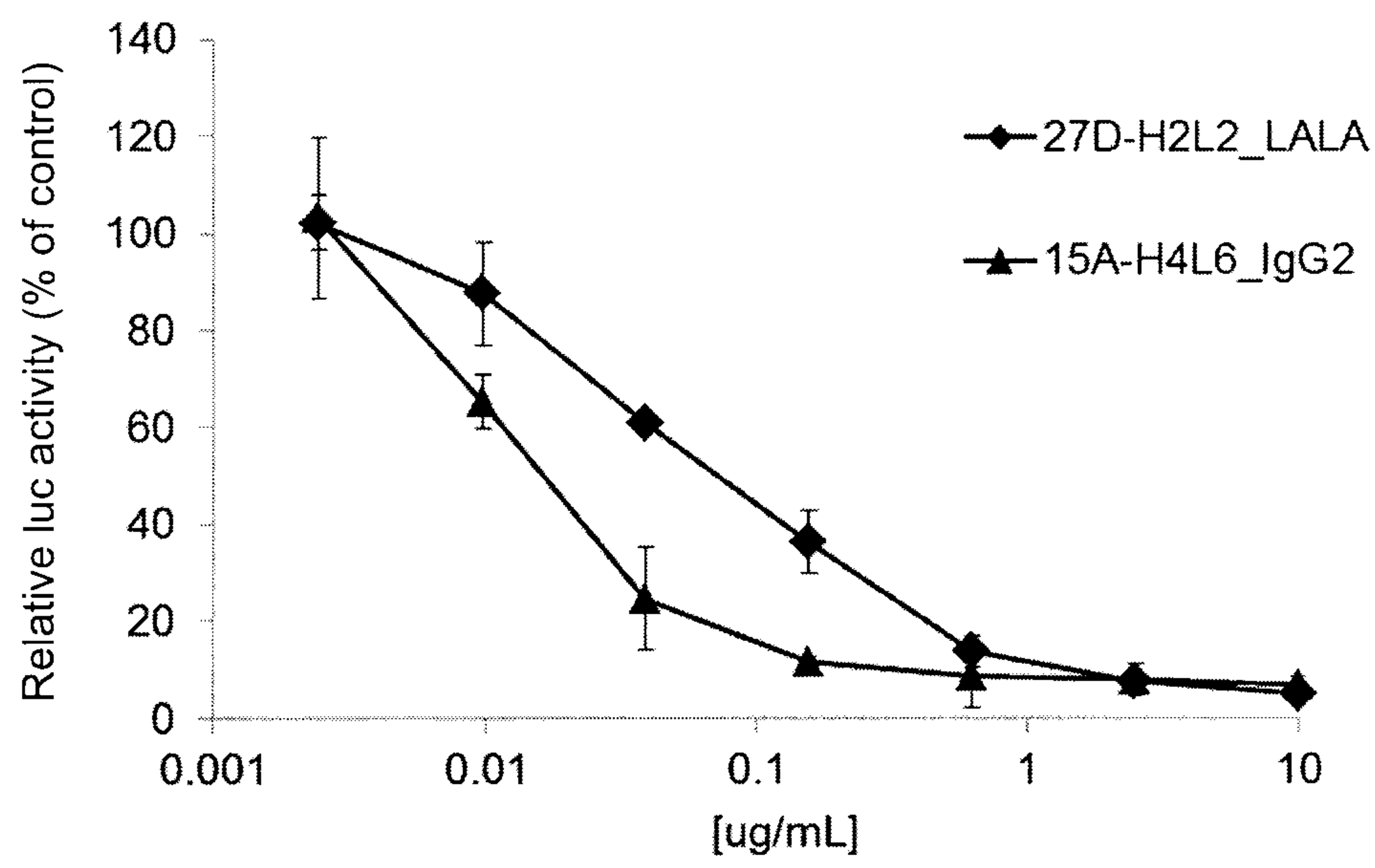


FIG. 36

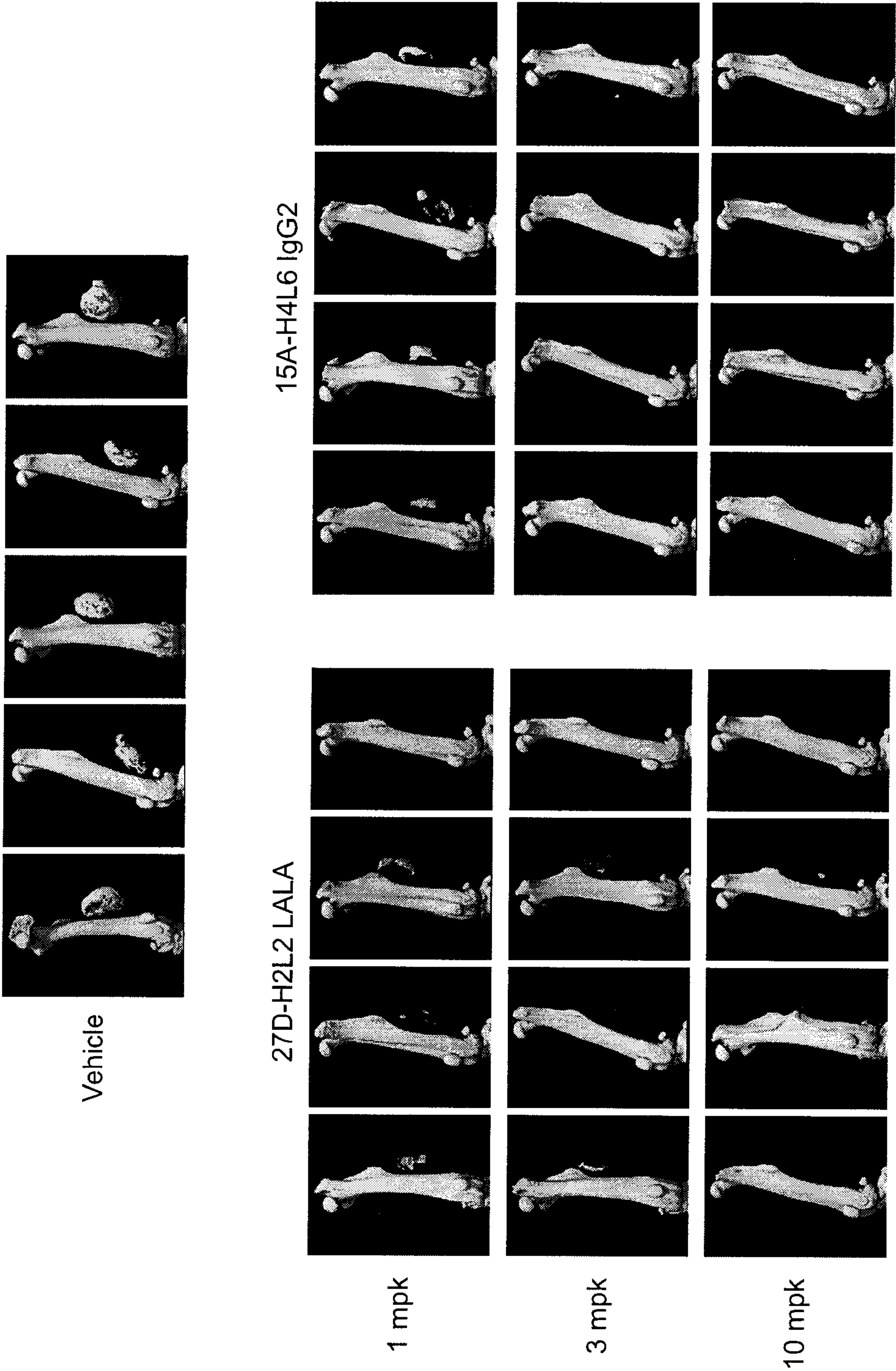


FIG. 37

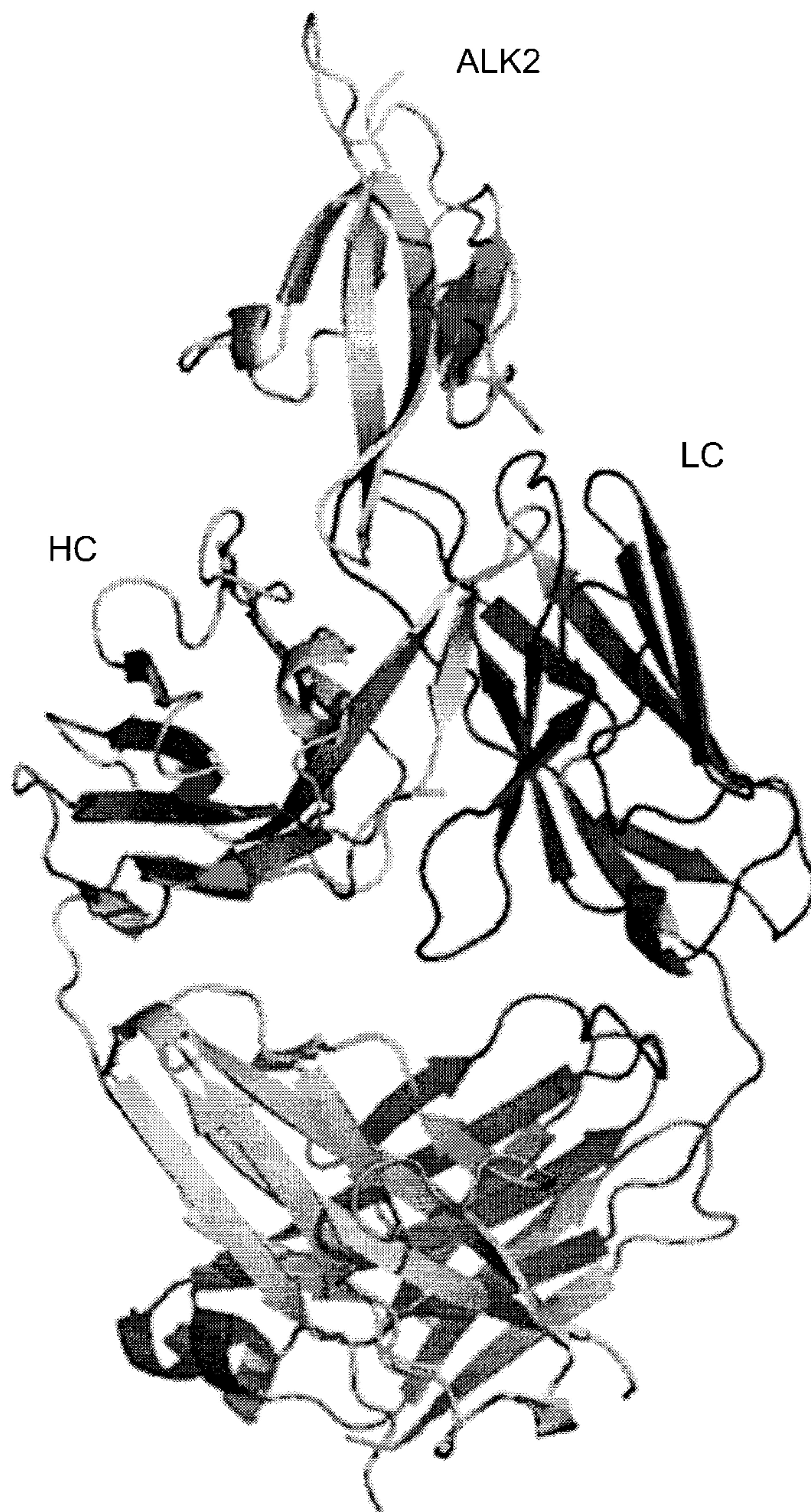


FIG. 38

SEQ ID NO:110: Nucleotide sequence of humanized hA2-11E-H3
ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCTTCACCTTCAG
CAACTACTACATGTACTGGATCCGGCAGGCCCCCTGGCAAGGGCCTGGAATGGGTGTCCAGCATCAACACCGAT
GGCGGCAGCACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCG
TGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGTGCCAAGAGCACCCCCAACAT
CCCCCTGGCCTATTGGGGCCAGGGAACCCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCAAGCGTCTTC
CCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCC
CCGAACCCGTGACCGTGAGCTGGAACACAGGCGCCCTGACCAGCGCGTGCACACCTTCCCCGCTGTCCTGCA
GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATC
TGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACACT
ACACATGCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTCTTCCCCC AAAACCCAA
GGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAG
GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGGAGCAGTACA
ACAGCACGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGCCAGCCCCGGGAA
CCACAGGTGTACACCCTGCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCA
AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCAC
CCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG
CAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCCAGAAGAGCCTCTCCC
TGTCTCCCGGCAAA
Signal sequence (1-57), Variable region (58-411), Constant region (412-1401)

SEQ ID NO:111: Amino acid sequence of humanized hA2-11E-H3
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSNYMYWIRQAPGKGLEWVSSINTD
GGSTYYADSVKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCAKSTPNIPLAYWGQGLTVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK
Signal sequence (1-19), Variable region (20-137), Constant region (138-467)

FIG. 39

SEQ ID NO:112: Nucleotide sequence of humanized hA2-11E-H4
ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCTTCACCTTCAG
CAACTACTACATGTACTGGATCCGGCAGGCCCCCTGGCAAGGGCCTGGAATGGATCAGCAGCATCAACACCGAC
GGCGGCAGCACCTACTACCCCGATAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCG
TGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGTGCCAAGAGCACCCCCAACAT
CCCCCTGGCCTATTGGGGCCAGGGAACCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCCAAGCGTCTTC
CCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCC
CCGAACCCGTGACCGTGAGCTGGAACCTCAGGCGCCCTGACCAGCGCGTGCACACCTTCCCCGCTGTCCTGCA
GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATC
TGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTC
ACACATGCCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTCTTCCCCC AAAACCCAA
GGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG
GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGGAGCAGTACA
ACAGCACGTACCGGGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGCCAGCCCCGGGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCA
AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACA ACTACAAGACCAC
CCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACC GTGGACAAGAGCAGGTGGCAG
CAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCCAGAAGAGCCTCTCCC
TGCTCTCCCGGCAAA

Signal sequence (1-57), Variable region (58-411), Constant region (412-1401)

SEQ ID NO:113: Amino acid sequence of humanized hA2-11E-H4
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSNYYMYWIRQAPGKGLEWISSINTD
GGSTYYPDSVKGRFTISRDN SKNTVY LQMNSLRAEDTAVYYCAKSTPN IPLAYWGQGLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK

Signal sequence (1-19), Variable region (20-137), Constant region (138-467)

FIG. 40

SEQ ID NO:114: Nucleotide sequence of humanized hA2-11E-L2
ATGGTGCTGCAGACCCAGGTGTTTCATCTCCCTGCTGCTGTGGATCTCCGGCGCGTACGGCGACATCCAGATGA
CCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTGACCATCACATGCAAGGCCAGCCAGAACAT
CTACAAGTACCTGAACTGGTTCCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACTACAGCAACAGC
CTGCAGACCGGCGTGCCCAGCAGATTTTCTGGCAGCGGCTCCGGCACCGACTTCACCCTGACAATCAGCTCCC
TGCAGCCCGAGGACTTCGCCACCTACTACTGCTTCCAGTACAGCAGCGGCCCCACCTTTGGCCAGGGCACCAA
GGTGGAAATCAAGCGTACGGTGGCCGCCCCCTCCGTGTTTCATCTTCCCCCCTCCGACGAGCAGCTGAAGTCC
GGCACCGCCTCCGTGGTGTGCCTGCTGAATAACTTCTACCCCAGAGAGGCCAAGGTGCAGTGGAAGGTGGACA
ACGCCCTGCAGTCCGGGAACTCCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGCACCTACAGCCTGAG
CAGCACCTGACCCTGAGCAAAGCCGACTACGAGAAGCACAAAGGTGTACGCCTGCGAGGTGACCCACCAGGGC
CTGAGCTCCCCCGTCACCAAGAGCTTCAACAGGGGGGAGTGT

Signal sequence (1-60), Variable region (61-384), Constant region (385-699)

SEQ ID NO:115: Amino acid sequence of humanized hA2-11E-L2
MVLQTQVFISLLLWISGAYGDIQMTQSPSSLSASVGDRVTITCKASQNIYKYLNWFQQKPGKAPKLLIYYSNS
LQTGVPSRFSGSGSGTDFLTIISSLQPEDFATYYCFQYSSGPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNRGEC

Signal sequence (1-20), Variable region (21-128), Constant region (129-233)

FIG. 41

SEQ ID NO:116: Nucleotide sequence of humanized hA2-11E-L3
ATGGTGCTGCAGACCCAGGTGTTTCATCTCCCTGCTGCTGTGGATCTCCGGCGCGTACGGCGACATCCAGATGA
CCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTGACCATCACATGCAAGGCCAGCCAGAACAT
CTACAAGTACCTGAACTGGTTCCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACTACAGCAACAGC
CTGCAGACCGGCATCCCCAGCAGATTTTCTGGCAGCGGCTCCGGCACCGACTTCACCCTGACAATCAGCTCCC
TGCAGCCCGAGGACTTCGCCACCTACTACTGCTTCCAGTACAGCAGCGGCCCCACCTTTGGCCAGGGCACCAA
GGTGGAAATCAAGCGTACGGTGGCCGCCCCCTCCGTGTTTCATCTTCCCCCCTCCGACGAGCAGCTGAAGTCC
GGCACCGCCTCCGTGGTGTGCCTGCTGAATAACTTCTACCCAGAGAGGCCAAGGTGCAGTGGAAGGTGGACA
ACGCCCTGCAGTCCGGGAACTCCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGCACCTACAGCCTGAG
CAGCACCTGACCCTGAGCAAAGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGC
CTGAGCTCCCCCGTCACCAAGAGCTTCAACAGGGGGGAGTGT
Signal sequence (1-60), Variable region (61-384), Constant region (385-699)

SEQ ID NO:117: Amino acid sequence of humanized hA2-11E-L3
MVLQTQVFISLLLWISGAYGDIQMTQSPSSLSASVGDRVTITCKASQNIYKYLNWFQQKPGKAPKLLIYYSNS
LQTGIPSRFSGSGSGTDFTLTISSLQPEDFATYYCFQYSSGPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNRGEC
Signal sequence (1-20), Variable region (21-128), Constant region (129-233)

FIG. 42

SEQ ID NO:118: Nucleotide sequence of humanized hA2-11E-L4
ATGGTGCTGCAGACCCAGGTGTTTCATCTCCCTGCTGCTGTGGATCTCCGGCGCGTACGGCGACATCCAGATGA
CCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGATAGAGTGACCATCAGCTGCAAGGCCAGCCAGAACAT
CTACAAGTACCTGAACTGGTTCCAGCAGAAGCCCGGCGAGGCCCCCAAGCTGCTGATCTACTACAGCAACAGC
CTGCAGACCGGCATCCCCAGCAGATTTTCTGGCAGCGGCTCCGGCACCGACTTCACCCTGACAATCAGCTCCC
TGCAGCCCGAGGACTTCGCTATCTACTTCTGTTTTCAATACTCCAGCGGCCCCACCTTCGGCCCTGGCACCAA
GGTGAAATCAAGCGTACGGTGGCCGCCCCCTCCGTGTTTCATCTTCCCCCCTCCGACGAGCAGCTGAAGTCC
GGCACCGCCTCCGTGGTGTGCCTGCTGAATAACTTCTACCCAGAGAGGCCAAGGTGCAGTGGAAGGTGGACA
ACGCCCTGCAGTCCGGGAACTCCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGCACCTACAGCCTGAG
CAGCACCTGACCCTGAGCAAAGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGC
CTGAGCTCCCCCGTCACCAAGAGCTTCAACAGGGGGGAGTGT
Signal sequence (1-60), Variable region (61-384), Constant region (385-699)

SEQ ID NO:119: Amino acid sequence of humanized hA2-11E-L4
MVLQTQVFISLLLWISGAYGDIQMTQSPSSLSASVGDRVTISCKASQNIYKYLNWFQQKPGEAPKLLIYYSNS
LQTGIPSRFSGSGSGTDFTLTISLQPEDFAIYFCFQYSSGPTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNRGEC
Signal sequence (1-20), Variable region (21-128), Constant region (129-233)

FIG. 43

SEQ ID NO:120: Nucleotide sequence of humanized hA2-25C-H3
ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCTTCACCTTCAG
CTACTACGCCATGAGCTGGGTGCGCCAGGCCCCCTGGAAAAGGCCTGGAATGGGTGGCCAGCATCAGCAGAGGC
GGCGACAACACCTACTACCGGGACAGCGTGAAGGGCCGGTTCACCACCAGCCGGGACAACAGCAAGAACACCC
TGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTATTGCGCCAGACTGAACTACAACAA
CTACTTCGACTACTGGGGCCAGGGCACCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCAAGCGTCTTC
CCCCCTGGCACCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCC
CCGAACCCGTGACCGTGAGCTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACACCTTCCCCGCTGTCCTGCA
GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATC
TGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTC
ACACATGCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTCTTCCCCC AAAACCCAA
GGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG
GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGGAGCAGTACA
ACAGCACGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGCCAGCCCCGGGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCA
AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACA ACTACAAGACCAC
CCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG
CAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCCAGAAGAGCCTCTCCC
TGTCTCCCGGCAAA

Signal sequence (1-57), Variable region (58-411), Constant region (412-1401)

SEQ ID NO:121: Amino acid sequence of humanized hA2-25C-H3
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSYYAMSWVRQAPGKGLEWVASISRG
GDNTYYRDSVKGRFTTSRDNSKNTLYLQMNSLRAEDTAVYYCARLNYN NYFDYWGQGLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK

Signal sequence (1-19), Variable region (20-137), Constant region (138-467)

FIG. 44

SEQ ID NO:122: Nucleotide sequence of humanized hA2-25C-H4
ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGCGAAGTGCAGCTGGTGG
AATCTGGCGGCGGACTGGTGCAGCCTGGCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCTTCACCTTCAG
CTACTACGCCATGAGCTGGGTGCGCCAGGCCCCCTGGAAAAGGCCTGGAATGGGTGGCCAGCATCAGCAGAGGC
GGCGACAACACCTACTACCGGGACAGCGTGAAGGGCCGGTTCACCACCAGCCGGGACAACAGCAAGAACACCC
TGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCACCTACTATTGCGCCAGACTGAACTACAACAA
CTACTTCGACTACTGGGGCCAGGGCGTGCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCAAGCGTCTTC
CCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCC
CCGAACCCGTGACCGTGAGCTGGAATCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCCGCTGTCCTGCA
GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATC
TGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTC
ACACATGCCCACCCTGCCCAGCACCTGAACTCCTGGGGGGACCCTCAGTCTTCCTCTTCCCCC AAAACCCAA
GGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG
GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGGAGCAGTACA
ACAGCACGTACCGGGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGCCAGCCCCGGGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCA
AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACA ACTACAAGACCAC
CCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG
CAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCCAGAAGAGCCTCTCCC
TGTCTCCCGGCAAA

Signal sequence (1-57), Variable region (58-411), Constant region (412-1401)

SEQ ID NO:123: Amino acid sequence of humanized hA2-25C-H4
MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSLRLSCAASGFTFSYYAMSWVRQAPGKGLEWVASISRG
GDNTYYRDSVKGRFTTSRDNSKNTLYLQMNSLRAEDTATYYCARLNYN NYFDYWQQV LVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK

Signal sequence (1-19), Variable region (20-137), Constant region (138-467)

FIG. 45

SEQ ID NO:124: Nucleotide sequence of humanized hA2-25C-L1
ATGGTGCTGCAGACCCAGGTGTTTCATCTCCCTGCTGCTGTGGATCTCCGGCGCGTACGGCGACATCCAGATGA
CCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTGACCATTACCTGTCAGGCCAGCCAGGACAT
CGGCAACTGGCTGAGCTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACGGCGCCACATCT
CTGGCCGATGGCGTGCCCGAGCAGATTTTCTGGCAGCGGCTCCGGCACCGACTTCACCCTGACAATCAGCAGCC
TGCAGCCCGAGGACTTCGCCACCTACTACTGTCTGCAAGCCTACAGCGCCCCCTTCACCTTTGGCCAGGGCAC
CAAGGTGGAAATCAAGCGTACGGTGGCCGCCCCCTCCGTGTTTCATCTTCCCCCCCCTCCGACGAGCAGCTGAAG
TCCGGCACCGCCTCCGTGGTGTGCCTGCTGAATAACTTCTACCCCAGAGAGGCCAAGGTGCAGTGGAAGGTGG
ACAACGCCCTGCAGTCCGGGAACTCCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGCACCTACAGCCT
GAGCAGCACCTGACCCTGAGCAAAGCCGACTACGAGAAGCACAAAGGTGTACGCCTGCGAGGTGACCCACCAG
GGCCTGAGCTCCCCCGTCACCAAGAGCTTCAACAGGGGGGAGTGT
Signal sequence (1-60), Variable region (61-387), Constant region (388-702)

SEQ ID NO:125: Amino acid sequence of humanized hA2-25C-L1
MVLQTQVFISLLLWISGAYGDIQMTQSPSSLSASVGDRVTITCQASQDIGNWLSWYQQKPGKAPKLLIYGATS
LADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQAYSAPFTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK
SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC
Signal sequence (1-20), Variable region (21-129), Constant region (130-234)

FIG. 46

SEQ ID NO:126: Nucleotide sequence of humanized hA2-25C-L2
ATGGTGCTGCAGACCCAGGTGTTTCATCTCCCTGCTGCTGTGGATCTCCGGCGCGTACGGCGACATCCAGATGA
CCCAGAGCCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTGACCATTACCTGTCAGGCCAGCCAGGACAT
CGGCAACTGGCTGAGCTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACGGCGCCACATCT
CTGGCCGATGGCGTGCCAGCAGATTTCAGCGGCAGCAGATCCGGCACCGACTACACCCTGACCATCAGCAGCC
TGCAGCCCGAGGACTTCGCCACCTACTACTGTCTGCAAGCCTACAGCGCCCCCTTCACCTTTGGCCAGGGCAC
CAAGGTGGAAATCAAGCGTACGGTGGCCGCCCCCTCCGTGTTTCATCTTCCCCCCTCCGACGAGCAGCTGAAG
TCCGGCACCGCCTCCGTGGTGTGCCTGCTGAATAACTTCTACCCCAGAGAGGCCAAGGTGCAGTGGAAGGTGG
ACAACGCCCTGCAGTCCGGGAACTCCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGCACCTACAGCCT
GAGCAGCACCTGACCCTGAGCAAAGCCGACTACGAGAAGCACAAAGGTGTACGCCTGCGAGGTGACCCACCAG
GGCCTGAGCTCCCCCGTCACCAAGAGCTTCAACAGGGGGGAGTGT
Signal sequence (1-60), Variable region (61-387), Constant region (388-702)

SEQ ID NO:127: Amino acid sequence of humanized hA2-25C-L2
MVLQTQVFISLLLWISGAYGDIQMTQSPSSLSASVGDRVTITCQASQDIGNWLSWYQQKPGKAPKLLIYGATS
LADGVPSRFSGSRSGTDYTLTISSLQPEDFATYYCLQAYSAPFTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK
SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEEKHKVYACEVTHQ
GLSSPVTKSFNRGEC
Signal sequence (1-20), Variable region (21-129), Constant region (130-234)

FIG. 47

SEQ ID NO:128: Nucleotide sequence of humanized hA2-25C-L3
ATGGTGCTGCAGACCCAGGTGTTTCATCTCCCTGCTGCTGTGGATCTCCGGCGCGTACGGCGACATCCAGATGA
CCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTGACCATTACCTGTCAGGCCAGCCAGGACAT
CGGCAACTGGCTGAGCTGGTATCAGCAGAAGCCCGGCAAGAGCCCCAAGCTGCTGATCTACGGCGCCACCTCT
CTGGCCGATGGCGTGCCAAGCAGATTCAGCGGCAGCAGATCCGGCACCCAGTACACCCTGACCATCAGCAGCC
TGCAGCCCCGAGGACTTCGCCACCTACTACTGTCTGCAAGCCTACAGCGCCCCCTTCACCTTTGGCAGCGGCAC
CAAGGTGGAAATCAAGCGTACGGTGGCCGCCCCCTCCGTGTTTCATCTTCCCCCCCCTCCGACGAGCAGCTGAAG
TCCGGCACCGCCTCCGTGGTGTGCCTGCTGAATAACTTCTACCCCAGAGAGGCCAAGGTGCAGTGGAAGGTGG
ACAACGCCCTGCAGTCCGGGAACTCCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGCACCTACAGCCT
GAGCAGCACCCCTGACCCTGAGCAAAGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAG
GGCCTGAGCTCCCCCGTCACCAAGAGCTTCAACAGGGGGGAGTGT
Signal sequence (1-60), Variable region (61-387), Constant region (388-702)

SEQ ID NO:129: Amino acid sequence of humanized hA2-25C-L3
MVLQTQVFISLLLWISGAYGDIQMTQSPSSLSASVGDRVTITCQASQDIGNWLSWYQQKPGKSPKLLIYGATS
LADGVPSRFSGRSGTQYTLTISSLQPEDFATYYCLQAYSAPFTFGSGTKVEIKRTVAAPSVFIFPPSDEQLK
SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC
Signal sequence (1-20), Variable region (21-129), Constant region (130-234)

FIG. 48

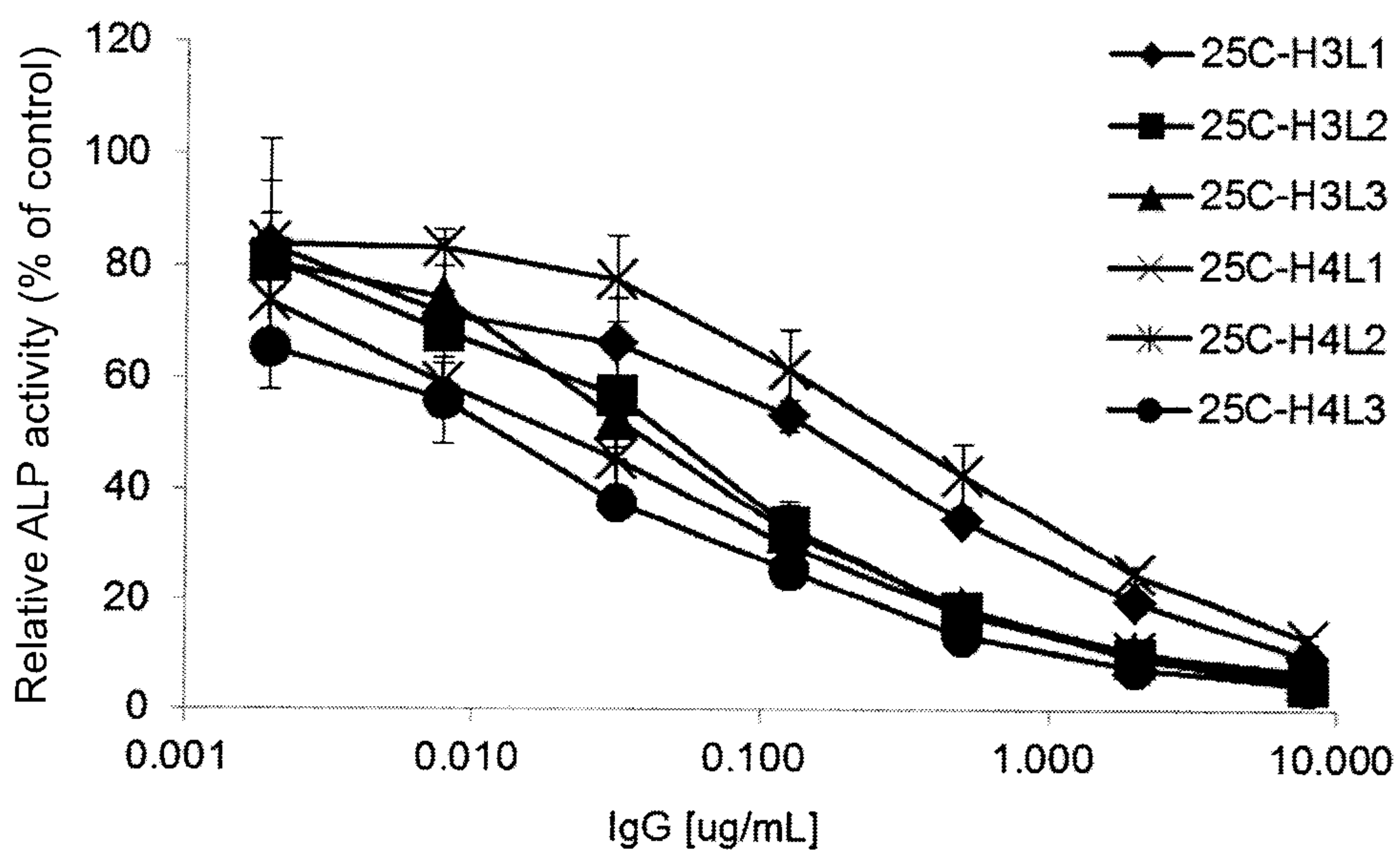
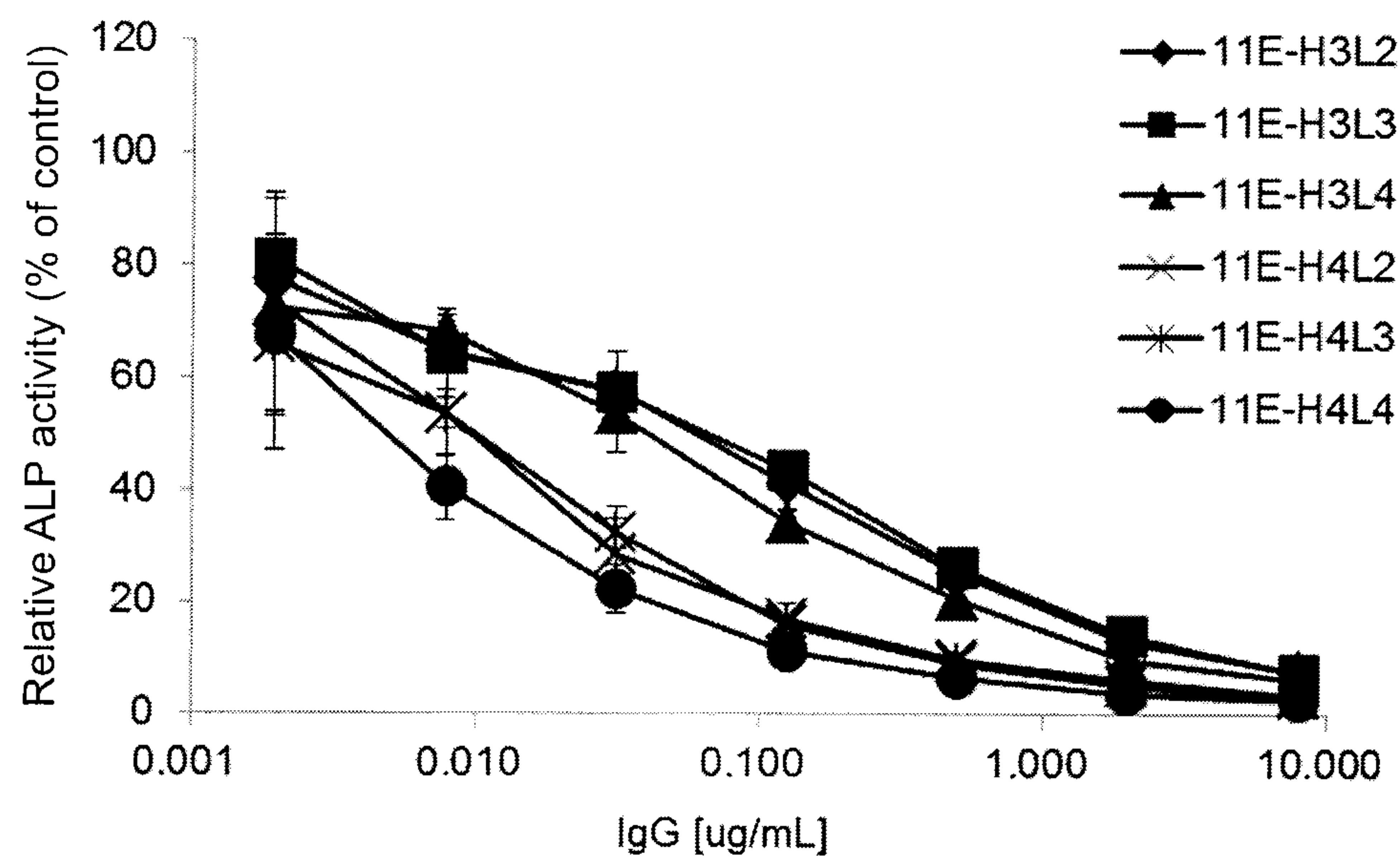


FIG. 49

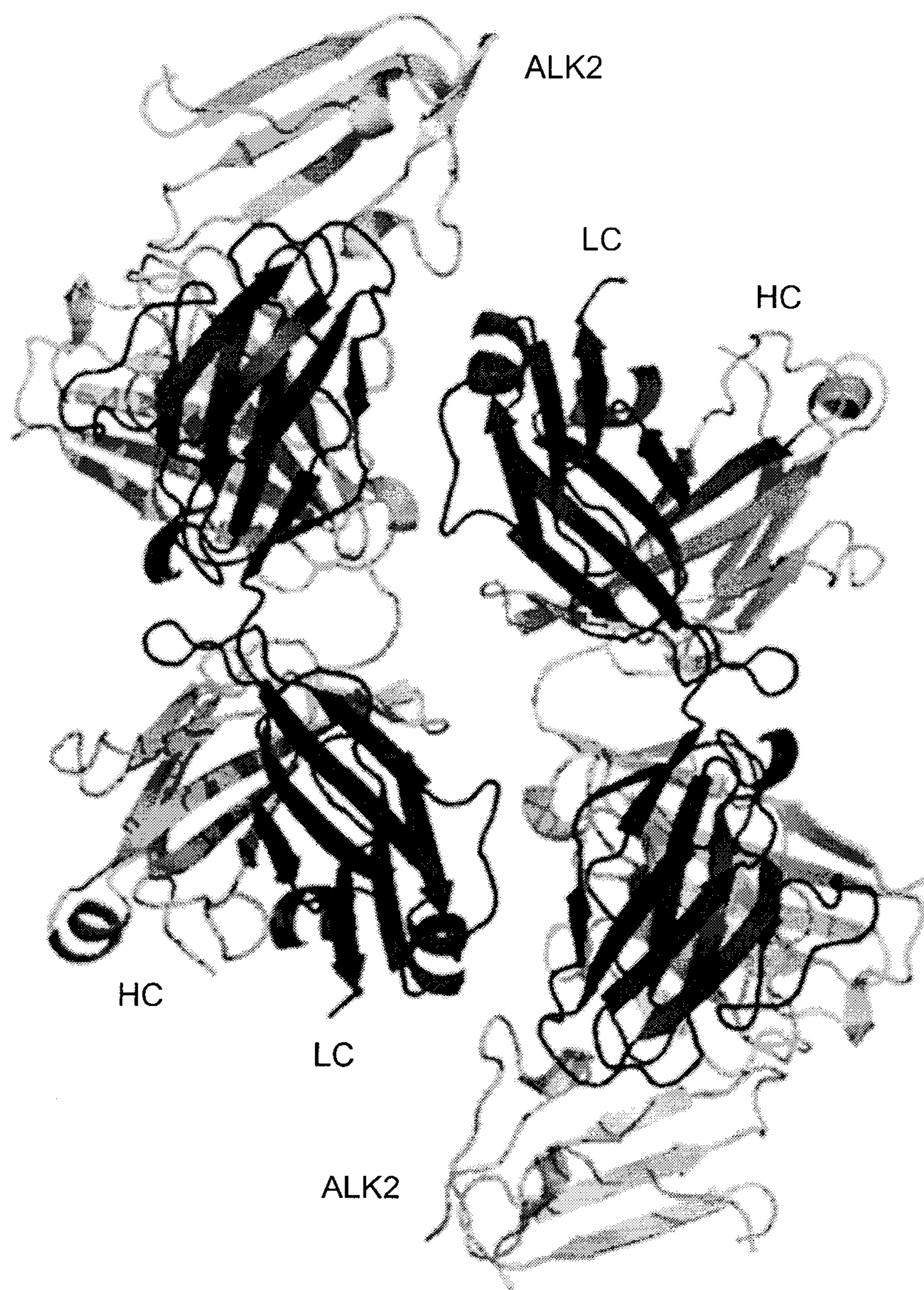
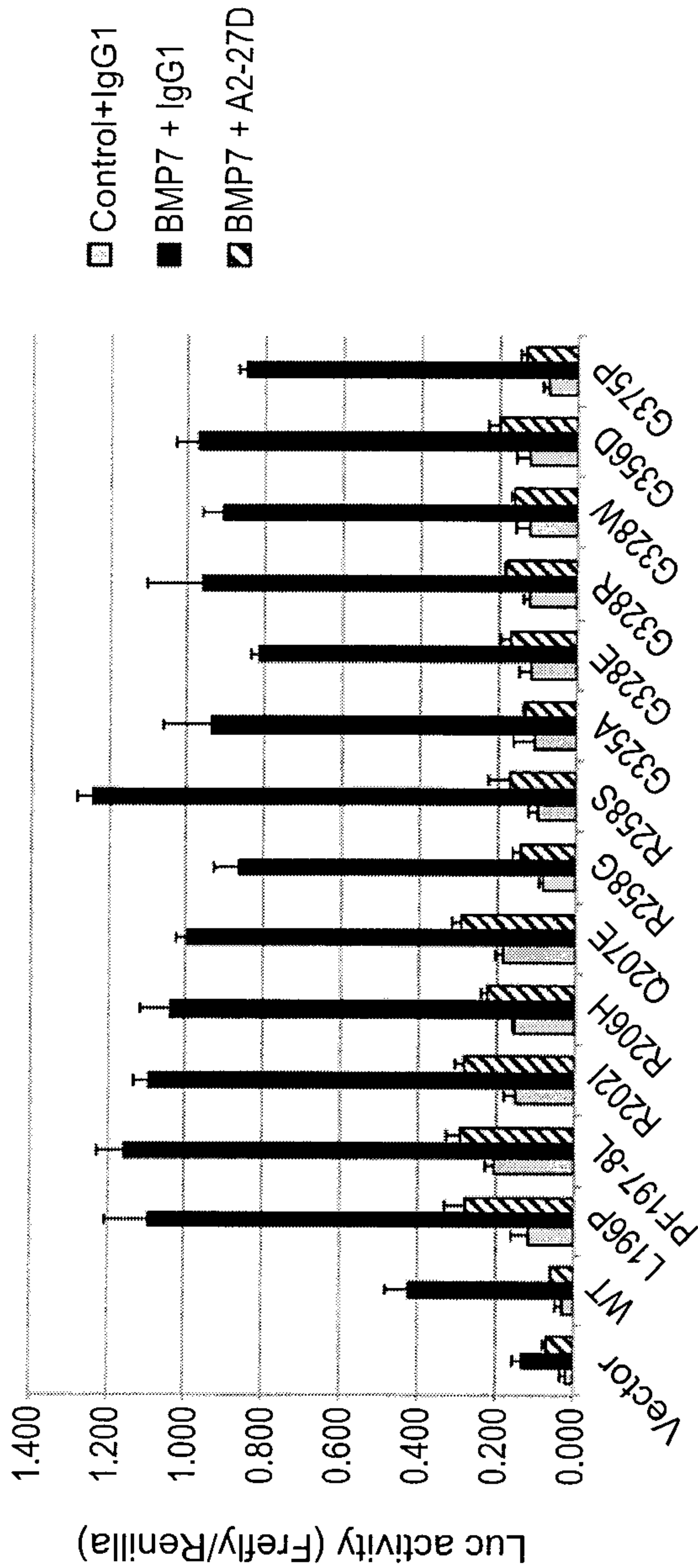


FIG. 50



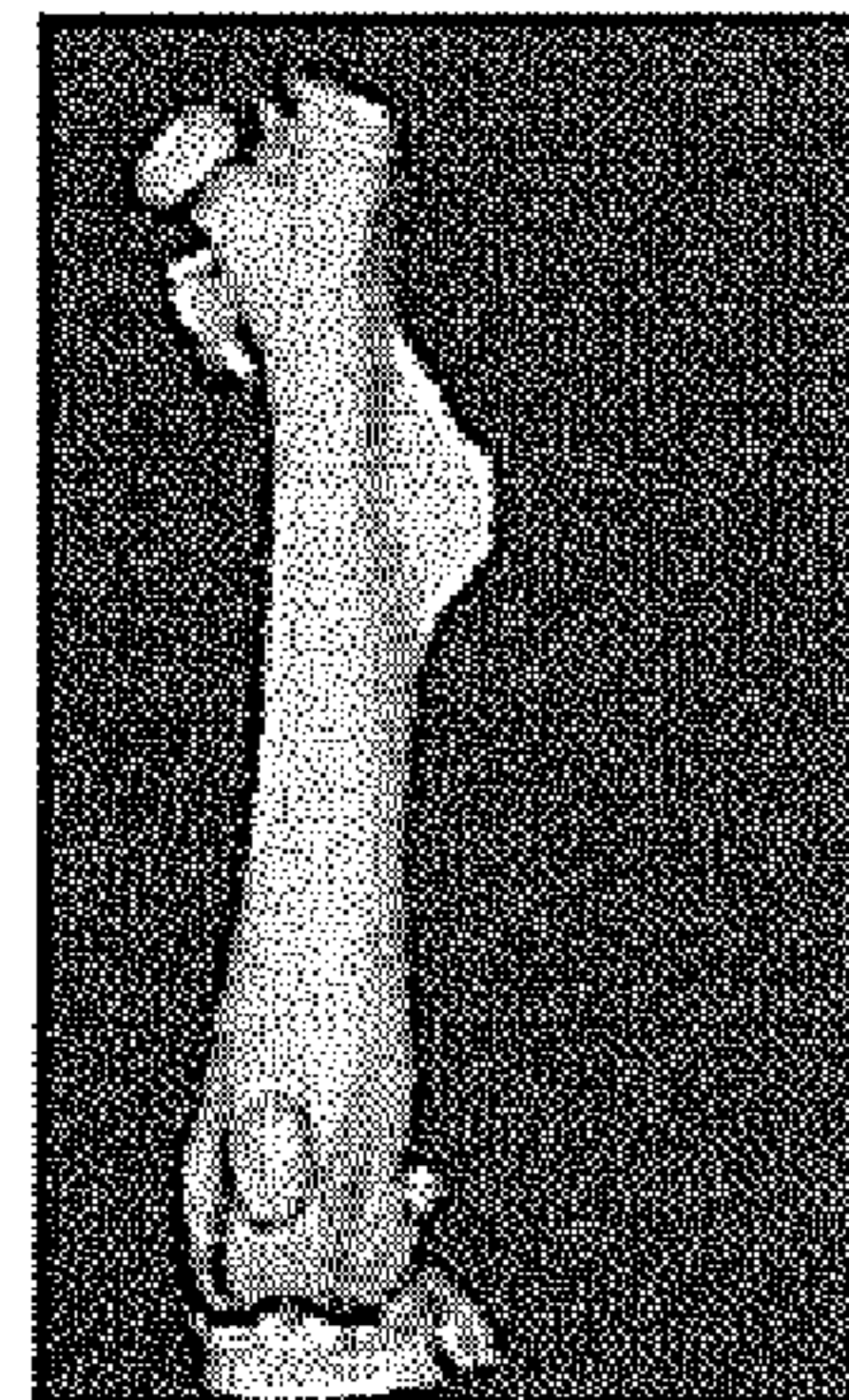
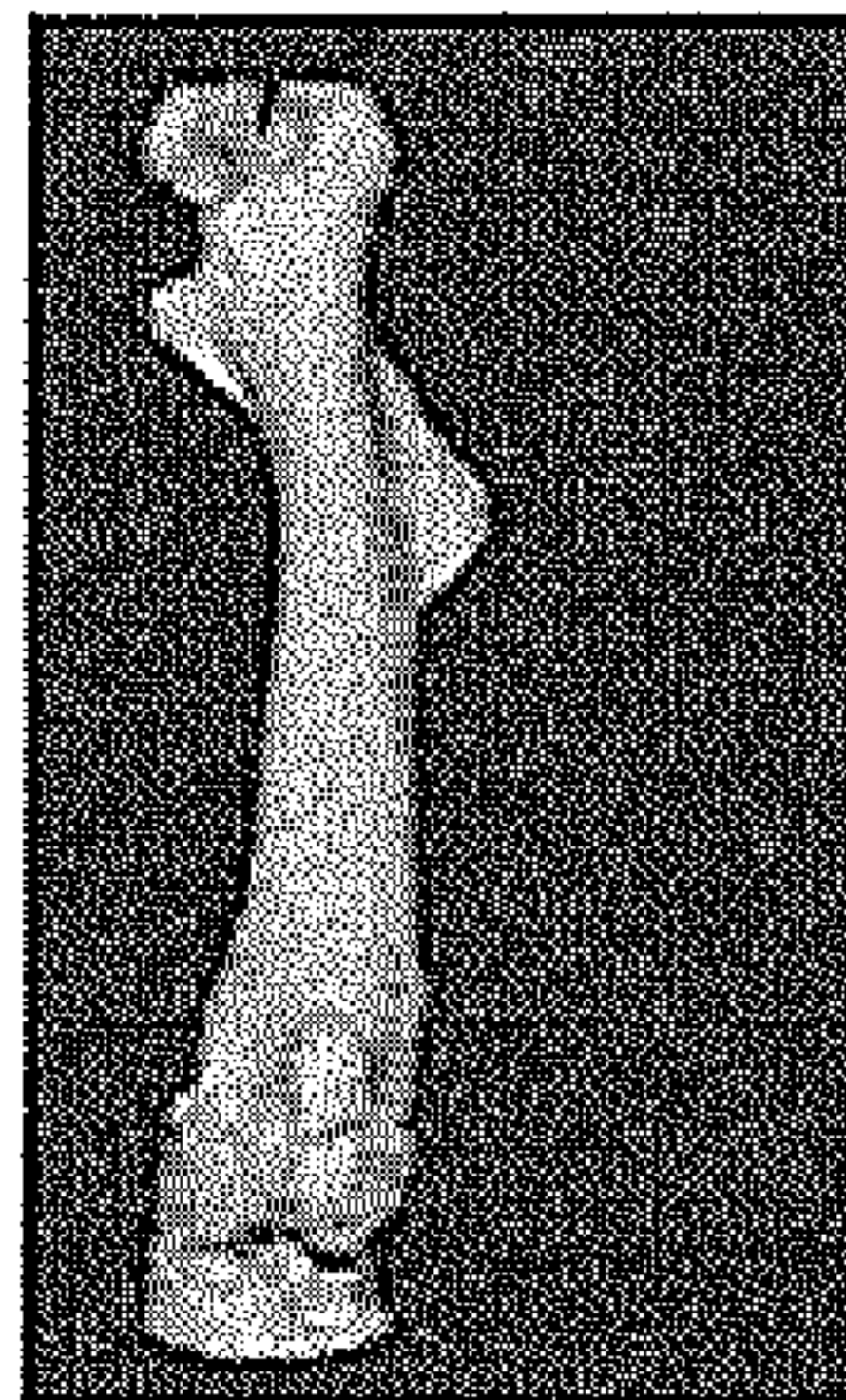
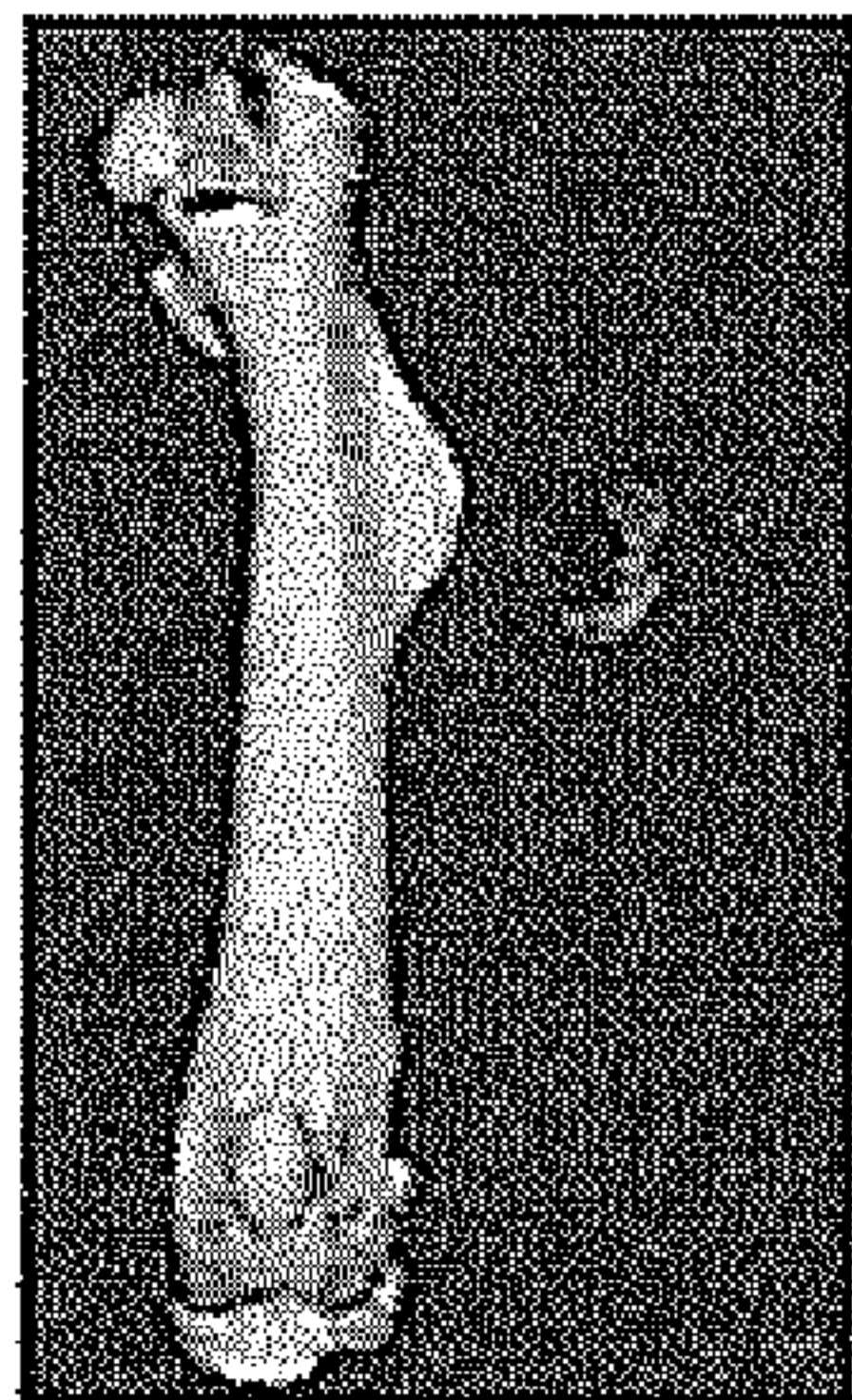
[図8]

AA
ビヒクル

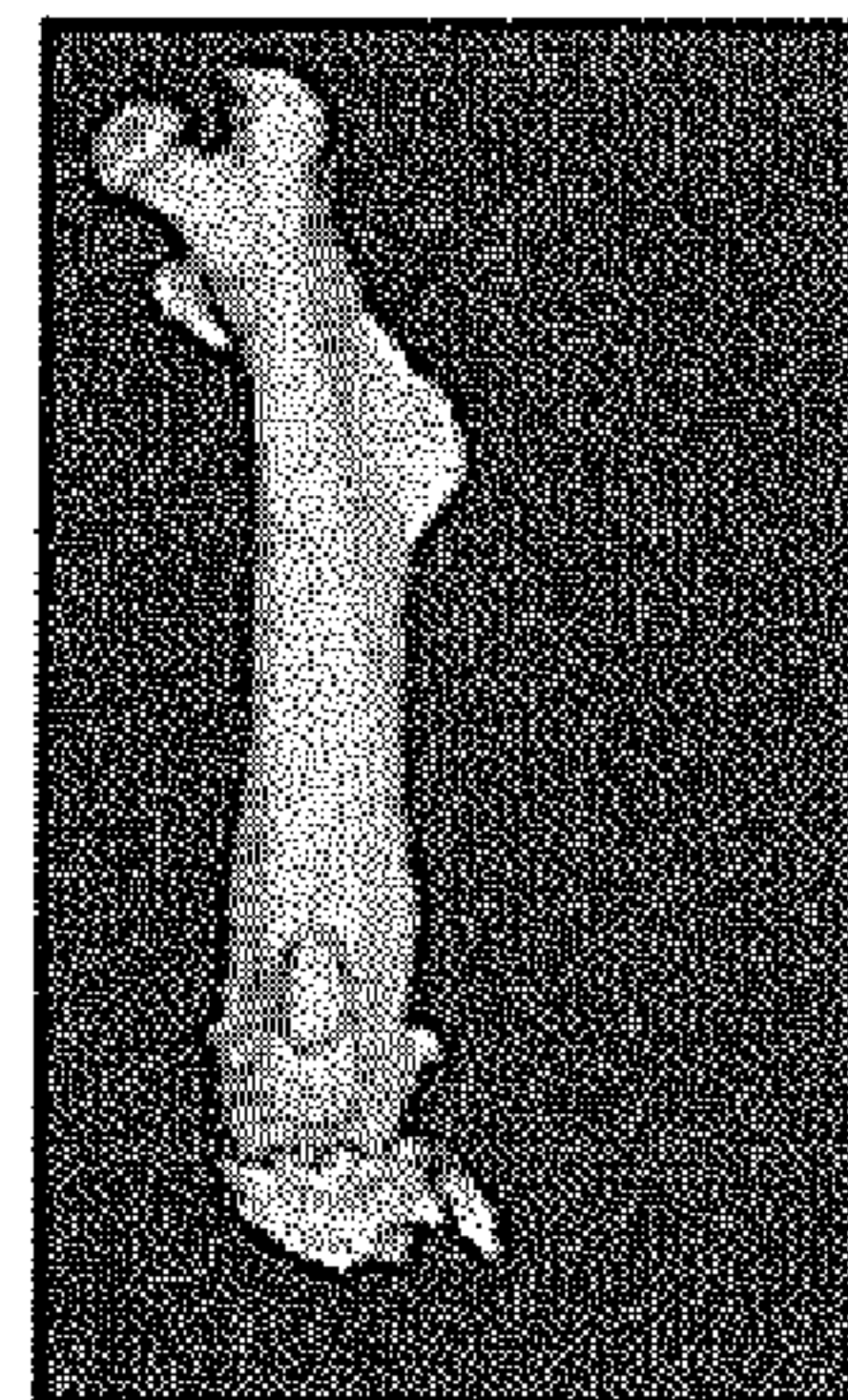
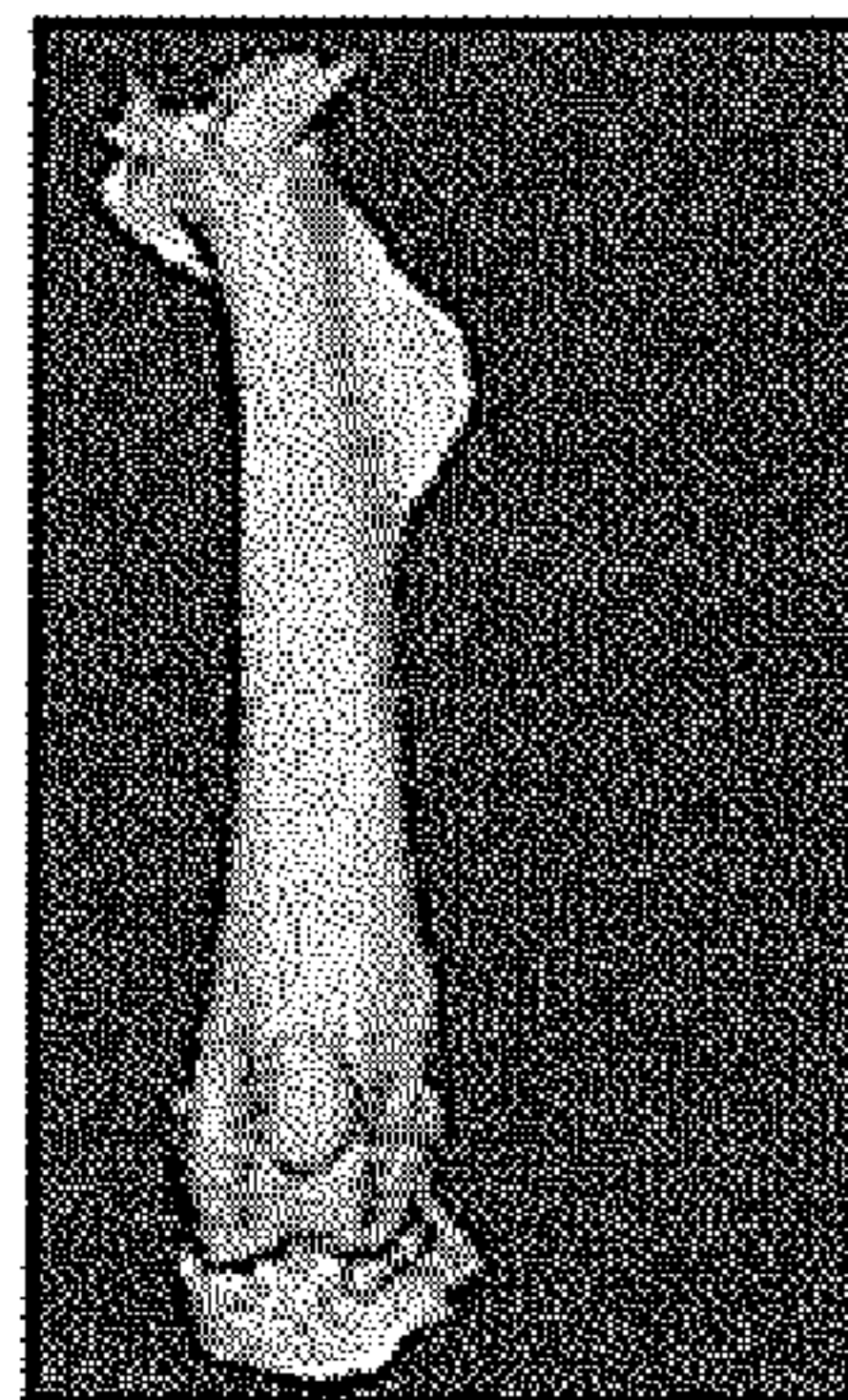
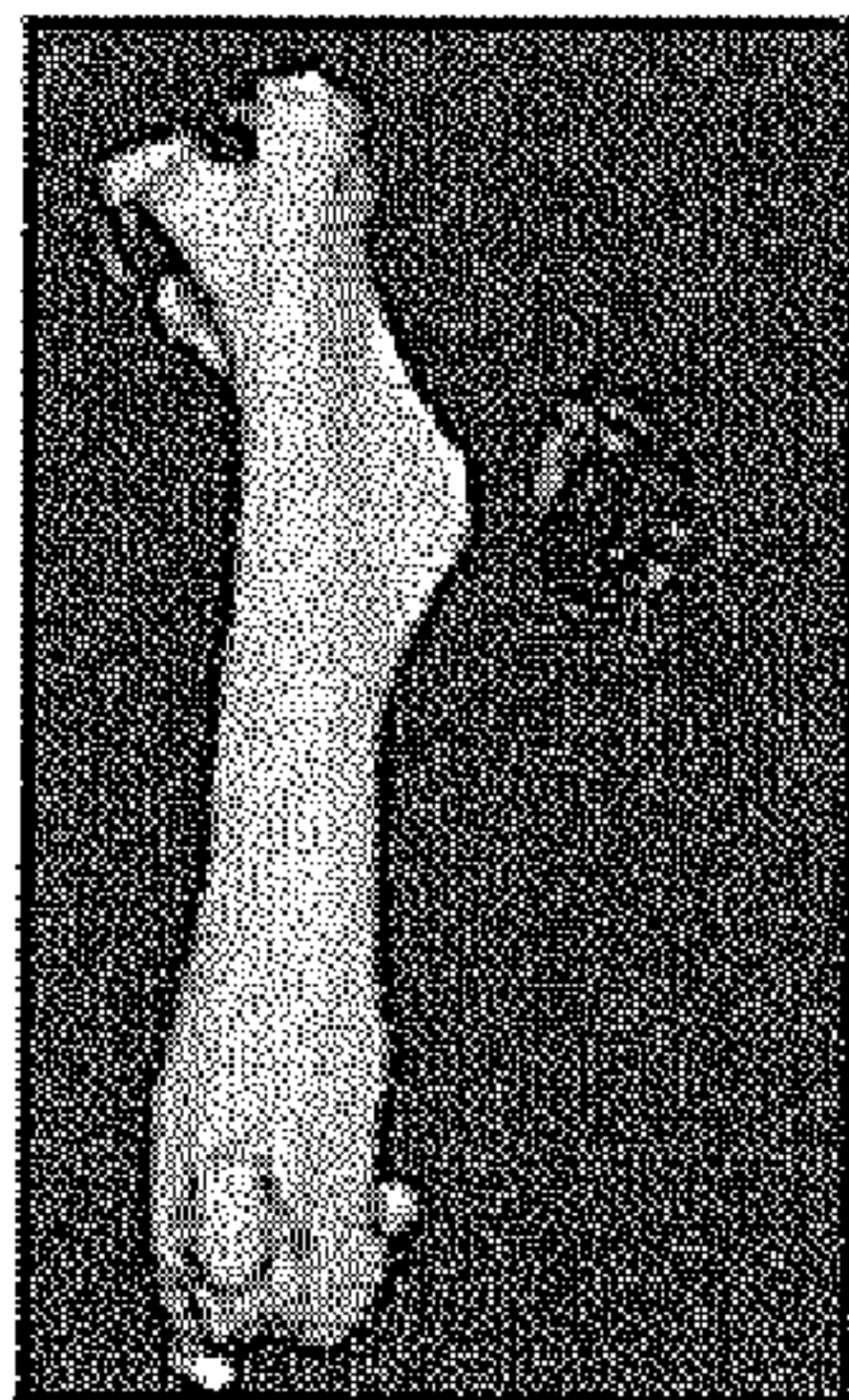
A2-15A

A2-27D

BMP7



BMP9



AA... VEHICLE