

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2012257254 B2**

(54) Title
Osteogenesis promoter

(51) International Patent Classification(s)
A61K 45/00 (2006.01) **A61P 19/10** (2006.01)
A61K 38/00 (2006.01) **A61P 29/00** (2006.01)
A61K 39/395 (2006.01) **A61P 43/00** (2006.01)
A61P 19/00 (2006.01) **G01N 33/15** (2006.01)
A61P 19/02 (2006.01) **G01N 33/53** (2006.01)
A61P 19/08 (2006.01) **C12N 15/09** (2006.01)

(21) Application No: **2012257254** (22) Date of Filing: **2012.05.11**

(87) WIPO No: **WO12/157237**

(30) Priority Data

(31) Number	(32) Date	(33) Country
2011-108642	2011.05.13	JP

(43) Publication Date: **2012.11.22**

(44) Accepted Journal Date: **2016.09.08**

(71) Applicant(s)
National University Corporation Tokyo Medical and Dental University

(72) Inventor(s)
Takayanagi, Hiroshi;Negishi, Takako

(74) Agent / Attorney
Spruson & Ferguson, L 35 St Martins Tower 31 Market St, Sydney, NSW, 2000

(56) Related Art
WO 2008/100995
CONROTTO P. et al, Sema4D induces angiogenesis through Met recruitment by Plexin B1, Blood, 2005;105:4321-4329

(12) 特許協力条約に基づいて公開された国際出願

(19) 世界知的所有権機関
国際事務局

(43) 国際公開日
2012 年 11 月 22 日(22.11.2012)



(10) 国際公開番号
WO 2012/157237 A1

- (51) 国際特許分類:
A61K 45/00 (2006.01) *A61P 19/10* (2006.01)
A61K 38/00 (2006.01) *A61P 29/00* (2006.01)
A61K 39/395 (2006.01) *A61P 43/00* (2006.01)
A61P 19/00 (2006.01) *G01N 33/15* (2006.01)
A61P 19/02 (2006.01) *G01N 33/53* (2006.01)
A61P 19/08 (2006.01) *C12N 15/09* (2006.01)
- (21) 国際出願番号: PCT/JP2012/003113
- (22) 国際出願日: 2012 年 5 月 11 日(11.05.2012)
- (25) 国際出願の言語: 日本語
- (26) 国際公開の言語: 日本語
- (30) 優先権データ:
特願 2011-108642 2011 年 5 月 13 日(13.05.2011) JP
- (71) 出願人 (米国を除く全ての指定国について): 国立
大学法人東京医科歯科大学(NATIONAL UNIVER-
SITY CORPORATION TOKYO MEDICAL AND
DENTAL UNIVERSITY) [JP/JP]; 〒1138510 東京都
文京区湯島一丁目 5 番 4 5 号 Tokyo (JP).
- (72) 発明者; および
- (75) 発明者/出願人 (米国についてのみ): 高柳 広
(TAKAYANAGI, Hiroshi) [JP/JP]; 〒1138510 東京都
文京区湯島一丁目 5 番 4 5 号国立大学法人東京
医科歯科大学内 Tokyo (JP). 根岸 貴子(NEGISHI,
Takako) [JP/JP]; 〒1138510 東京都文京区湯島一丁
目 5 番 4 5 号国立大学法人東京医科歯科大学内
Tokyo (JP).
- (74) 代理人: 廣田 雅紀(HIROTA, Masanori); 〒
1070052 東京都港区赤坂二丁目 8 番 5 号若林ビ
ル 3 階 Tokyo (JP).
- (81) 指定国 (表示のない限り、全ての種類の国内保
護が可能): AE, AG, AL, AM, AO, AT, AU, AZ, BA,
BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO,
CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS,
JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS,
LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX,
MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT,
QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST,
SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VC, VN, ZA, ZM, ZW.
- (84) 指定国 (表示のない限り、全ての種類の広域保
護が可能): ARIPO (BW, GH, GM, KE, LR, LS, MW,
MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), ユーラシ
ア (AM, AZ, BY, KG, KZ, RU, TJ, TM), ヨーロッパ
(AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR,
GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT,
NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG).
- 添付公開書類:
— 国際調査報告 (条約第 21 条(3))
— 明細書の別個の部分として表した配列リスト
(規則 5.2(a))

(54) Title: OSTEOGENESIS PROMOTER

(54) 発明の名称: 骨形成促進剤

(57) Abstract: The purpose of the present invention is to provide an osteogenesis promoter for directly promoting osteogenesis by osteoblasts, and an agent for preventing and treating bone disease. The present invention is characterized in that a binding inhibitor substance of semaphorin 4D and plexin B1 is used. For the binding inhibitor substance, suitable examples include anti-semaphorin 4D antibody, anti-plexin B1 antibody, and protein comprising the extracellular domain of plexin B1.

(57) 要約: 本発明は、骨芽細胞による骨形成を直接促進する骨形成促進剤や、骨疾患の予防・治療剤を提供することを目的とするものである。セマフォリン 4D とプレキシン B1 との結合阻害物質を用いることを特徴とする。かかる結合阻害物質としては、抗セマフォリン 4D 抗体、抗プレキシン B1 抗体、プレキシン B1 の細胞外領域を含むタンパク質などを好適に例示することができる。



WO 2012/157237 A1

OSTEOGENESIS PROMOTER

[Technical Field]

[0001]

The present invention relates to an osteogenesis accelerator and to a preventive and therapeutic agent. It relates more particularly to an osteogenesis accelerator and to a preventive and therapeutic agent having as active ingredient a substance which inhibits binding of semaphorin 4D and plexin B1.

[Background of Invention]

[0002]

As Japan's population ages, there has been an increase in patients suffering from bone fractures, osteoporosis, articular rheumatism, lumbar pain and other bone diseases. The function of tissue in bone tissue is maintained by coordinating osteoblasts which support osteogenesis and osteoclasts which support bone resorption and by maintaining a balance between osteogenesis and bone resorption. The balance of bone metabolism is destroyed by aging, a decline in ovarian function as well as other factors. When osteogenesis decreases or when there is abnormal bone resorption the bone quantity (bone density) decreases and a variety of bone diseases occur. Bone diseases include bone fractures, bone deficiency, osteoporosis, osteomalacia, osteopenia, lumbar pain, Paget's disease of bone, tonic myelitis, articular rheumatism, deformative arthrosis and the like. In particular, when an elderly person is afflicted with a bone disease, it is difficult for that person to function on a level required for daily life and depending on the case, there is a risk that the patient will become bed-ridden. As a result, this is extremely significant in the prevention and treatment of bone diseases in modern society where an increasing number of people are elderly.

[0003]

Activated vitamin D3, biphosphonate, calcitonin, hormone preparations containing estradiol, vitamin K2 preparations and the like are generally used as therapeutic agents for bone diseases. Estradiol derivatives, activated vitamin D3 derivatives, biphosphonate derivatives and the like are being developed as more effective therapeutic agents as they have fewer side effects (see Patent Document 1). However, vitamin D3 has an action which increases the concentration of calcium in the blood and estradiol and biphosphonate have a bone resorption inhibition effect. However, none of these has an effect which directly accelerates osteogenesis through the osteoblasts. A multiple drug therapy is the treatment of choice in this case so that there is a need to develop a novel preventive and therapeutic agent having a different action from that of the conventional preventive and therapeutic agents.

[0004]

Bones are being recreated continuously in stages known as bone remodeling wherein the bone resorption stage and the following osteogenesis stage are repeated. The transition from one stage to another must be controlled precisely by secretion from the bone cell as it contributes to the communication between the osteoclast-osteoblast or by bone reconstruction factors released from the bone matrix. It is well known that TGF- β and IGF-1 released during bone resorption stimulate osteogenesis so that it is known as a coupling factor. Although there is an abundance of *in vitro* data on candidate molecules which are extremely important for the coupling factor, there is still no *in vitro* evidence for this.

[0005]

Axon guidance molecules manifest widely outside the nervous system. Therefore, cell wandering, the immune response, tissue development and angiogenesis and the like are controlled (see Non-Patent Documents 1 and 2). Based on research carried out in recent years, it is suggested that the axon guidance molecules of semaphorin and ephrin and the like contribute to intercellular communication between osteoclasts and osteoblasts (see Non-Patent Documents 3 through 5).

[0006]

It is well known that Semaphorin 4D is secreted from oligodendrocytes and that it induces destruction of the growth cone in the central nervous system. It is also clear that semaphorin 4D is extremely important in maintaining the immune response and the homeostasis of the immune system. One well-known semaphorin 4D receptor is plexin-B1 (see Non-Patent Document 6). Furthermore, Non-Patent Document 7 suggests that osteoclast formation can be accelerated through the osteoblast differentiation inhibition action as well as the osteoblasts. Non-Patent Document 8 discloses that semaphorin 4D is not detected in the osteoblasts, that it is present on the surface of the osteoclasts and the bone quantity has been confirmed to increase as compared to wild-type mice in female sema4D^{-/-} mice where Semaphorin 4D (Sema 4D) is deficient in homo [zygotes].

[Prior Art Documents]

[Patent Documents]

[0007]

Special Table in Publication 2005-509629.

[0008]

[Non-Patent Document 1] B.J. Dickson, Science 298, 1959 (Dec 6, 2002)

[Non-Patent Document 2] A.B. Huber, A.L. Kolodkin, D.D. Ginty, J.F. Cloutier, Annual Review Neuroscience 26, 509 (2003)

- [Non-Patent Document 3] N. Takagahara et al., Nat. Cell Biol 8,615 (Jun, 2006)
- [Non-Patent Document 4] N. Irie et al., Cell Metab 4, 111 (Aug, 2006)
- [Non-Patent Document 5] C. Zhao et al., Cell Metab 4, 111 (Aug, 2006)
- [Non-Patent Document 6] I. Oinuma et al., Science, Vol. 305, No. 5685, pp. 862 -865 (August 6, 2004)
- [Non-Patent Document 7] Ishida Masanari, Kaneda Toshio, Muto Akihiro, Yoshida Masashi, Meeting Program Abstracts of the Japan Society of Bone Metabolism, vol. 25, page 266, published June 2007, [Analysis of Bioaction in Bone Metabolism in Osteoclast-derived Semaphorin 4D]
- [Non-Patent Document 8] Romain Dacquin, Chantal Domenget, Pierre Jurdic, Irma Machuca-Gayet, ASBMR 2010 Annual Meeting Abstracts, Presentation Abstracts, Presentation Number MOO152, "Physiological Control of Bone Resorption by Semaphorin 4D is Dependent on Ovarian Function"

[Overview of Invention]

[0009]

It is an aspect of the present invention to provide an osteogenesis acceleration agent and a preventive and therapeutic agent for bone diseases which involves direct promotion of osteogenesis using osteoblasts.

[0010]

After a great deal of hard work and research under the conditions described in the abovementioned background of technology, the inventors found that (a) when semaphorin 4D which is derived from osteoclasts binds with the plexin B receptor on the osteoblast, it activates the small G protein RhoA which inhibits osteoblast differentiation, decreases the IRS signals (signals which promote the differentiation of the osteoblasts) and inhibit the differentiation of the osteoblasts so that osteogenesis is inhibited; and that (b) the anti-semaphorin 4D antibodies and anti-plexin B1 antibodies directly accelerate osteogenesis using the osteoblasts.

[0010a]

According to one aspect the present invention provides a method for the prevention and/or treatment of bone diseases comprising administering to a subject in need thereof a therapeutically effective amount of an agent comprising as an active ingredient a substance which inhibits binding of semaphorin 4D and plexin B1.

[0010b]

According to one aspect the present invention provides a method for determining a candidate active ingredient for an osteogenesis acceleration agent comprising determining whether a substance being studied inhibits binding between semaphorin 4D and plexin B1, wherein if the substance inhibits said binding, the substance is determined to be a candidate active ingredient for an osteogenesis acceleration agent.

[0010c]

According to one aspect the present invention provides use of a substance which inhibits binding of semaphoring 4D and plexin B1 in the manufacture of a medicament for the prevention and/or treatment of bone diseases.

[0011]

This means that the present invention relates to (1) an osteogenesis acceleration agent having as active ingredient a substance which inhibits binding between semaphorin 4D and plexin B1; (2) an osteogenesis acceleration agent as described in (1) above characteristic in that the binding inhibiting substance is an anti-semaphorin 4D antibody; and (3) an osteogenesis acceleration agent as described in (1) above characteristic in that the binding inhibiting substance is an anti-plexin B1 antibody or a protein containing plexin B1 extracellular regions.

[0012]

The present invention also relates to (4) an agent for prevention and treatment of bone diseases having as active ingredient a substance which inhibits binding of semaphorin 4D and plexin B1; (5) an agent for prevention and treatment of bone diseases as described in (4) above characteristic in that the binding inhibition substance is an anti-semaphorin4D antibody; (6) an agent for prevention and treatment of bone diseases as described in (4) above characteristic in that the binding inhibition substance is an anti-plexin B1 antibody or a protein containing plexin B1 extracellular regions; and (7) an agent for prevention and treatment of bone diseases as described in any of (4) through (6) above characteristic in that the bone diseases are selected from bone fracture, bone deficiency, osteoporosis, osteomalacia, osteopenia, lumbar pain, Paget's disease of bone, tonic myelitis, articular rheumatism and deformative arthrosis.

[0013]

The present invention also relates to (8) a method used to determine a candidate active ingredient for an osteogenesis acceleration agent wherein it is determined whether a substance being studied is a substance which inhibits binding between semaphorin 4D and plexin B1, and if the abovementioned substance being studied is the abovementioned substance which inhibits binding, the abovementioned substance being studied is determined to be a candidate active ingredient for an osteogenesis acceleration agent; (9) a method as described in (8) above characteristic in that the method used to determine whether a substance being studied is a substance which inhibits binding between semaphorin 4D and plexin B1 consists of the following steps (A) through (D): (A) a step wherein semaphorin 4D and plexin B1 are brought in contact with one another in the presence of the substance being studied, (B) a step wherein the degree of binding between semaphorin 4D and plexin B1 is measured, (C) a step wherein the degree measured in step (B) above is compared with the degree when not in the presence of the substance being studied, and (D) a step wherein the substance being studied is determined to be a substance which inhibits binding between semaphorin 4D and plexin B1 when the degree measured in step (B) above is lower than the degree when not in the presence of the substance being studied; and (10) a method of screening for a candidate active ingredient for an osteogenesis acceleration agent characteristic in that the methods described in (8) or (9) above are used to search substances being studied for a candidate active ingredient for an osteogenesis acceleration agent.

[0014]

The present invention can be used to directly promote osteogenesis using osteoblasts and to prevent and/ or treat bone diseases.

[Brief Explanation of Figures]

[0015]

[Figure 1] A diagram indicating bone quantity in wild mice (WT) measured using microcomputer tomography (μ CT) and semaphorin 4D knockout (Sema 4d^{-/-}) mice.

[Figure 2] A diagram indicating the trabecular width in WT mice measured using μ CT and Sema4d^{-/-} mice.

[Figure 3] A diagram indicating osteogenesis [surface area of osteoblast (left);, surface area of calcified bone (center); and osteogenesis rate (right)] measured using osteomorphogenesis analysis in WT mice and Sema4d^{-/-} mice with calcein double marker every 4 days.

[Figure 4] A diagram indicating the osteoclast surface area and the cell count (at left and in the middle) as well as the parameters (at right) of the bone resorption measured using osteomorphogenesis analysis in WT mice and Sema4d^{-/-} mice.

[Figure 5] A diagram indicating the bone quantity of mice in which adoptive immune cell grafting has been carried out using the bone marrow cells of WT mice and Sema4d^{-/-} mice. The second graph from the left indicates the bone quantity of mice when grafted onto WT mice and the second graph from the right indicates the bone quantity of mice when grafted onto Sema4d^{-/-} mice.

[Figure 6] A diagram indicating the number of bone nodes when Fc-sema4D (recombined Semaphorin 4D fused with the Fc area of the IgG1) are added to the calvarial cells cultured under the osteogenesis conditions.

[Figure 7] A diagram indicating the manifestation of Plexin B type and mRNA of cluster of differentiation 72 (CD72) during osteoblast differentiation.

[Figure 8] A diagram indicating results of pull down analysis using Fc-sema4D. The panel on the right indicates results of sampling analysis prior to pull down and the panel on the left indicates results of sampling analysis after pull down.

[Figure 9] A diagram indicating the bone quantity in WT mice measured using μ CT analysis and in Plexin B1 knockout (Plxnb1^{-/-}) mice.

[Figure 10] A diagram indicating the width of the trabecula in WT mice measured using μ CT mice and Plxnb1^{-/-} mice.

[Figure 11] A diagram indicating the surface area of osteoblasts in WT mice measured using osteomorphogenesis analysis.

[Figure 12] A diagram indicating bone surface area which has become calcified in WT mice measured using osteomorphogenesis analysis and Plxnb1^{-/-} mice.

[Figure 13] A diagram indicating the osteogenesis rate in WT mice measured using osteomorphogenesis analysis and Plxnb1^{-/-} mice.

[Figure 14] A diagram indicating RA mutants (R1661/1662/1968A) of Plexin B1 and Plexin B1 having mutations in the GAP domain as well as the mutant cut for Plexin B1 in which the PDZ bond domain is missing (Δ PDZ).

[Figure 15] A diagram indicating the bone quantity in WT mice (control mice) measured using μ CT analysis and dominant negative RhoA (RhoA DNOB) mice.

[Figure 16] A diagram indicating the trabecula in WT mice measured using μ CT analysis and RhoA DNOB.

[Figure 17] A diagram indicating the formation [osteoblast surface area] in WT mice and RhoA DNOB mice measured using osteomorphogenesis analysis.

[Figure 18] A diagram indicating the formation [surface area of calcified bone] of WT mice and RhoADNOB mice.

[Figure 19] A diagram indicating the formation [osteogenesis rate] in WT mice and RhoA DNOB mice measured using osteomorphogenesis analysis.

[Figure 20] A diagram indicating the surface area of osteoblasts of mice with extracted ovaries (OVX) and OVX mice treated with anti-Sema4D bodies measured using osteomorphogenesis analysis.

[Figure 21] A diagram indicating the bone quantity of OVX mice and OVX mice treated with anti-semaphorin 4D antibodies (anti-Sema4D antibodies) measured using μ CT analysis.

[Figure 22] A diagram indicating the osteogenesis [osteogenesis rate] in OVX mice and in OVX mice treated with anti-Sema4D antibodies measured using osteomorphogenesis analysis.

[Figure 23] A diagram indicating the trabecular intervals in OVX mice and in OVX mice treated with anti-Sema4D antibodies measured using μ CT analysis.

[Figure 24] The upper panel in Figure 24 is a diagram indicating results of studying the calcification formation of mice osteoblasts cultured using osteoclasts of WT mice of Sema4D-/- mice and when cultured in the presence of anti-Sema4D antibodies. The lower panel in Figure 24 is a diagram indicating the results of studying the calcification of mouse osteoblasts when cultured in the presence of Fc-sema4d and / or anti-plexin Ba antibodies.

[Figure 25] A diagram indicating results of studying the calcification formation of human osteoblasts (HOS) cultured in the presence of osteoclasts or the supernatant and / or the anti-Sema4D antibodies and the like.

[Figure 26] A diagram indicating the surface area of osteoblasts of mice with extracted ovaries (OVX) and OVX mice treated with anti-semaphorin 4D antibodies (anti-Sema4D antibodies) 6 weeks after OVX treatment measured using osteomorphogenesis analysis.

[Figure 27] A diagram indicating the bone quantity of OVX mice and OVX mice treated with anti-Sema4D antibodies 6 weeks after OVX treatment measured using μ CT analysis.

[Figure 28] A diagram indicating the osteogenesis [osteogenesis rate] in OVX mice and in OVX mice treated with anti-Sema4D antibodies 6 weeks after OVX treatment measured using osteomorphogenesis analysis.

[Figure 29] A diagram indicating the trabecular intervals in OVX mice and in OVX mice treated with anti-Sema4D antibodies 6 weeks after OVX treatment measured using μ CT analysis.

[Preferred Mode of Working Invention]

[0016]

There are no particular restrictions on the [osteogenesis accelerator] in the present invention and on the [preventive and therapeutic agent for bone diseases] (hereinafter referred to collectively as [agent in the present invention], as long as they have as active ingredient a substance which inhibits binding of semaphorin 4D and plexin B1 (hereinafter, referred to simply as [binding inhibiting substance in the present invention]) as the active ingredient and there are no particular restrictions on the binding inhibitor in the present invention as long as it is a substance which inhibits binding of semaphorin 4D and plexin B1 of vertebrates. However,

protein comprising the extracellular regions of anti-semaphorin 4D antibodies, anti-plexin B1 antibodies, and plexin B1 can be cited as suitable examples. The binding inhibitor in the present invention inhibits binding of semaphorin 4D derived from osteoclasts and plexin B1 receptors on the osteoblasts and by inhibiting suppression of differentiation of osteoblasts through RhoA activation inhibition and through inhibition of lowering of IRS signals so that it is thought to accelerate osteogenesis using osteoblasts. Furthermore, in this Specification, the terms [inhibition] and [suppression] are used interchangeably.

[0017]

The abovementioned anti-semaphorin 4D antibodies and anti-plexin B1 antibodies (hereinafter referred to as [antibodies in this invention]) may be polyclonal antibodies and may be monoclonal and any functional fragment of these. However, monoclonal antibodies are preferred given their high specificity. The abovementioned anti-semaphorin 4D antibodies and anti-plexin B1 antibodies may be produced using a conventional well-known method using semaphorin 4D and plexin B1. The functional fragments which are the antibodies in the present invention indicate fragments of antibodies which bind specifically relative to (a) semaphorin 4D which is an antigen in which the antibodies in the present invention bind specifically and to (b) plexin B. Specific examples are F(ab')₂, Fab', Fab, Fv, disulphide-linked FV, single-chain FV (scFV) and polymers of these (D.J. King, Applications and Engineering of Monoclonal Antibodies, 1998 T.J. International Ltd.). These antibody fragments can be obtained by using a conventional method such as digestion of antibodies molecules using papain, pepsin and other proteases or by using a well-known genetic engineering method.

[0018]

The antibodies in the present invention also include human antibodies. Here, [human antibodies] as these relate to the present invention indicate antibodies which are manifested products of human-derived antibody genes. Human antibodies can be obtained by introducing a human antibody gene locus and administering semaphorin 4D and plexin B1 in transgenic animals which are capable of producing human-derived antibodies. A mouse is an example of said transgenic animal. Mice which are capable of producing human antibodies, for example, are deficient in endogenous mouse immunoglobulin (Ig) heavy chains and mouse κ light chains and mice can be used which simultaneously retain a 14th chromosome fragment (SC20) comprising the human Ig heavy chain genes as well as human Ig κ chain transgenes (KCo5). These mice are produced by breeding strain A mice having a human Ig heavy chain gene locus and strain B mice having a human Ig κ chain transgene. The strain A mice are homozygotes for both endogenous Ig heavy chain and κ light chain destruction and they are a mouse strain (Tomizuka, et al., Proc Natl Acad Sci USA, 200 Vol 97:722) which retain a 14th chromosome fragment (SC20) which is capable of apomorphic transmission. Strain B is also a homozygote for both endogenous mouse Ig heavy chains and κ light chain deficiency and is a mouse strain (Nat Biotechnol., 1996 Vol 14:845) which retains a human Ig κ chain transgene (KCo5).

[0019]

The polyclonal antibodies in the present invention may be produced using the method indicated below. They can be obtained by using semaphorin 4D and plexin B1 and if necessary an immunoactivation agent (Freund's adjuvant and the like) on mice, rabbits, goats, horses and other non-human mammals. The monoclonal antibodies in the present invention are used to produce hybridoma from antibody producing cells from immunosensitized animals and myeloma cells which cannot produce their own antibodies. The hybridoma is cloned and clones are selected which produce monoclonal antibodies indicating specific affinity toward the antigens used for immunization. Said hybridoma can be produced based on the method of Keller and Millstein (Nature, 1975 Vol. 256:495-497). Screening of the hybridoma clone which produces monoclonal antibodies is carried out by culturing the hybridoma in a micro-titer plate. The responsiveness to the immunoantigens in the culturing supernatant in the wells seen to have proliferated can be carried out by measuring using ELISA and other enzyme immunoassay methods, radioimmunoassay, the fluorescent antibody method and other immunological methods.

[0020]

Producing monoclonal antibodies from hybridomas can be carried out by culturing the hybridoma *in vitro* and isolating it from the supernatant. It can also be cultured *in vivo* in the pleural fluid of mice, rats, guinea pigs, hamsters or rabbits and the like and isolated from the pleural fluid. The genes which code monoclonal antibodies from hybridoma and other antibody producing cells are cloned, recombined in a suitable vector and these are introduced in the host (for example, chinese hamster ovary (CHO) cells and other mammalian cell stock, *Escherichia coli*, yeast cells, insect cells, plant cells and the like) and the recombinant antibodies are produced using gene recombination technology (P.J. Delves, Antibody Production Essential Techniques, 1997, Wiley, P. Shepherd and C. Dean, Monoclonal Antibodies, 2000, Oxford University Press, J.W. Goding, Monoclonal Antibodies: Principles and Practice, 1993, Academic Press).

[0021]

Transgenic cows, goats, sheep or pigs wherein the desired antibody genes which are recombined in endogenous genes using transgenic animal production technology are produced and large quantities of antibodies derived from antibody genes are obtained from the milk of these transgenic animals.

[0022]

The antibodies produced can be refined using a well-known method in the field such as a combination of protein A column chromatography, ion exchanged chromatography, hydrophobic

chromatography, the sulfate analytical method, gel filtration, affinity chromatography and the like.

[0023]

The protein comprising the extracellular region of the abovementioned plexin B1 can inhibit binding of semaphorin 4D and plexin B1 by trapping semaphorin 4D. There are no particular restrictions on the protein which comprises the plexin B1 extracellular region, however, a protein which fuses with the constant region of the antibodies (preferably any immunoglobulin Fc fragment) is a suitable example.

[0024]

The protein which comprises the abovementioned semaphorin 4D, plexin B1 and plexin B1 extracellular regions can produce a manifestation vector which comprises that sequence based on the sequence information of these proteins. The manifestation vector is subjected to phenotypic transformation in suitable host cells, the target protein is produced inside the cells and the target protein can be introduced by using an isolating method or other well-known method. For example, the DNA sequence³ (sequence number 1) of human semaphorin 4D and the amino acid sequence (sequence number 2) are disclosed in GenBank Accession Number NM_001142287 and the DNA sequence (sequence number 3) of human plexin B1 and the amino acid sequence (sequence number 4) are disclosed in GenBank Accession Number NM_001130082. Furthermore, the human plexin B1 extracellular region corresponds to amino acid numbers 1 through 1490 of the amino acid sequence of the abovementioned Accession Number NM_001130082.

[0025]

We measured by immunoblotting analysis the binding between semaphorin 4D and plexinB1 both in the presence of and not in the presence of the substance to see whether a certain substance is a substance which inhibits binding of semaphorin 4D and plexinB1 and studied whether the bond between them decreased in the presence of that substance so that it could be easily confirmed.

[0026]

The osteogenesis acceleration effect referred to in the present invention indicates the effect of accelerating osteogenesis and more precisely includes the effect of accelerating osteogenesis by osteoblasts by inhibiting the suppression of differentiation of osteoblasts. Whether or not a certain substance has an osteogenesis acceleration effect can be confirmed by administering the substance to vertebrates having a lower bone quantity than usual (preferably patients with osteoporosis and osteoporosis model vertebrates) and finding whether or not the bone quantity increases.

[0027]

The bone disease prevention and treatment effect in the present invention indicates the effect of preventing and / or treating any of the bone diseases or the effect of improving the symptoms in the present invention. Whether or not a certain substance has a bone disease therapeutic effect can be confirmed by administering the substance to a patient or a vertebrate with a bone disease (preferably a patient with osteoporosis or an osteoporosis model vertebrate) and then studying whether the bone disease is cured or improved.

[0028]

The formulation of the present invention may contain only the binding inhibition substance in the present invention, however, a usually pharmacologically permitted carrier, binding agent, stabilizer, excipient, diluent, pH buffer, disintegrator, solubilizing agent, solubilization adjuvant, isotoner and other adjustment agent compounding components may be added. The formulation of the reducing agent in the present invention may be a powder formulation, granulated formulation, a capsule agent or other solid preparation. It may also be a solution preparation, an emulsion, a suspension or other liquid formulation. These preparations may be used as suitable for the binding inhibition substance in the present invention by treating using the regular method.

[0029]

There are no particular restrictions on the method used to administer the agent in the present invention as long as it has the desired bone disease preventive or therapeutic effect and it may be administered orally or non-orally. The non-oral method used to administer it includes vascular administration, muscular administration, hypodermic administration, transdermal administration, nasal administration, transpulmonary administration. Of these, vascular and intravenous administration can be used to particular advantage. The dose of the preparation in the present invention as well as the number of times administered and the concentration can be adjusted in accordance with the body weight of the subject, the type of bone disease and the bone disease symptoms.

[0030]

The subject for the present invention may be vertebrates such as mammals and animals belonging to the bird family. These may include humans, monkeys, mice, rats, hamsters, guinea pigs, cows, pigs, horses, rabbits, sheep, goats, cats, dogs, chickens, quail and other suitable animals. Of these, humans and domestic animals and fowl are particularly suitable. The binding inhibiting substance contained in the agent of the present invention is suitable from the standpoint of obtaining an outstanding osteogenesis acceleration effect and the bone disease prevention and treatment effect whether the type of vertebrate derived from semaphorin 4D and plexin B1 which bring out the binding inhibition action coincides with the type of vertebrate which is a candidate for administration of the agent in the present invention. Furthermore, a

vertebrate derived from semaphorin 4D and the type of vertebrate derived from plexin B1 may be the same or different.

[0031]

There are no particular restrictions on the type of bone disease which can be treated using the present invention as long as it is a bone disease having a factor which brings about a decline in osteogenesis or a bone disease related to a decline in osteogenesis. However, bone fractures, bone deficiency, osteoporosis, osteomalacia, bone deficiency, lumbar pain, Paget's disease of bone, tonic myelitis, articular rheumatism, dysosteogenesis and deformative arthrosis are all suitable candidates. Of these, osteoporosis, osteomalacia, osteopenia and dysosteogenesis are particularly suitable.

[0032]

The method in the present invention is a method used to determine a candidate active ingredient for an osteogenesis acceleration agent wherein it is determined whether a substance being studied is a substance which inhibits binding between semaphorin 4D and plexin B1, and if the abovementioned substance being studied is the abovementioned substance which inhibits binding, the abovementioned substance being studied is determined to be a candidate active ingredient for an osteogenesis acceleration agent. In the abovementioned determination method, the following steps (A) through (D) are a suitable example of the method used to determine whether a substance being studied is a substance which inhibits binding between semaphorin 4D and plexin B1:

- (A) a step wherein semaphorin 4D and plexin B1 are brought in contact with one another in the presence of the substance being studied;
- (B) a step wherein the degree of binding between semaphorin 4D and plexin B1 is measured;
- (C) a step wherein the degree measured in step (B) above is compared with the degree when not in the presence of the substance being studied;
- (D) a step wherein the substance being studied is determined to be a substance which inhibits binding between semaphorin 4D and plexin B1 when the degree measured in step (B) above is lower than the degree when not in the presence of the substance being studied.

[0033]

In the abovementioned determination method, the following may be used to measure the degree of binding: for semaphorin 4D, labeled semaphorin 4D extracellular region or fusion protein of semaphorin 4D extracellular region and immunoglobulin Fc region, etc., and for plexin B1, a cell expressing plexin B1 on its surface. The following may also be used to measure the degree of binding: for plexin B1, labeled plexin B1 extracellular region or fusion protein of plexin B1 extracellular region and immunoglobulin Fc region, etc., and for semaphorin 4D, a cell expressing semaphorin 4D on its surface.

[0034]

There are no particular restrictions on the substance being studied in the determination method in the present invention and it may be a substance predetermined to have an activity which inhibits binding of semaphorin 4D and plexin B1 and it may be any substance whose activity is unknown. It is also possible to simultaneously use multiple substances being studied. When simultaneously using multiple substances being studied, it is possible to simultaneously use individual substances being studied each in a separate sample, to simultaneously use multiple substances being studied in a single sample, or to simultaneously use multiple samples individually prepared with multiple substances being studied. When simultaneously using multiple substances being studied in a single sample, it may not be possible in one test to determine which of the substances being studied inhibits binding of semaphorin 4D and plexin B1, but it is possible to determine which of the substances being studied is a binding inhibitor by running the test multiple times and narrowing down the substances being studied in stages. The determination method in the present invention is characterized by searching substances being studied for a candidate active ingredient for an osteogenesis acceleration agent, and the method can be used to screen for a candidate active ingredient for an osteogenesis acceleration agent.

[0035]

Furthermore, other modes of the present invention may involve (a) use of the binding inhibition substance in the present invention in the production of the preventive and therapeutic agent for the abovementioned osteogenesis acceleration agent and bone diseases; (b) a binding inhibition substance in the present invention for use as an osteogenesis acceleration agent and in the prevention and treatment of bone diseases; (c) a method of using the binding inhibition substance for the acceleration of osteogenesis and prevention and treatment of bone diseases; (d) a method of promoting osteogenesis by administering the binding inhibition substance in the present invention; and (e) a method of preventing and treating bone diseases by administering the binding inhibition substance in the present invention.

[0036]

Next we shall describe the present invention in detail referring to practical examples of it, however, it should by no means be construed that the present invention is restricted to these practical examples.

[Practical Examples 1]

[0037]

[Analysis of Mice and Bone Phenotypes]

We produced the Sema4d^{-/-}, Plxnb1^{-/-}, plexin B2 knockout (Plxnb2^{-/-}), CAT-RhoA DN and α 1 (I)-Cre mice in accordance with the method described in the literature (W. Shi *et al.*, Immunity 13, 633 (Nov, 2000); R. H. Friedel *et al.*, J Neurosci 27, 3921 (Apr 4, 2007); R.H. Friedel *et al.*, Proc Natl Acad Sci USA 102, 13188 (Sep 13, 2006); K. Kobayashi *et al.*, J.

Neurosci 24, 3480 (Apr 7, 2004); R. Dacquin, M. Starbuck, T. Schinke; G. Karsenty, Dev Dyn 224, 245 (Jun, 2002)]. All of the mice were backcrossed 8 or more times with C57BL/6 mice. All of the mice were maintained in a condition where there was no specific pathogenic fungus. All of the animal experiments were authorized by the Animal Experiment Committee of the Tokyo Medical and Dental University and complied with related guidelines and laws. Analysis of the bone phenotype involved controlling respectively the genetically altered mice and the mice from the same litter and we analyzed at least 8 males and 8 females. Three-dimensional micro CT (μ CT) analysis and histomorphological measurement analysis were carried out in accordance with the methods described in the literature (K. Nishikawa *et al.*, J Clin Invest 120, 3455 (Oct 1, 2010); T. Koga *et al.*, Nature 428, 758 (Apr 15, 2004).

[0038]

[Bone Marrow Chimera Mice]

We produced bone marrow chimera mice by changing in part the method described in the literature (B. Zhao *et al.*, Nat Med 15, 1066 (Sep, 2009). This means that we collected donor bone marrow cells (C57BL6-Ly5, 2 backgrounds) from wild-type *Sema4d*^{-/-} mice from the same litter. We injected intravenously 2×10^6 cells obtained from each donor in the caudal vein of wild type recipient mice (3 weeks old, C57BL/6-Ly5, 1 background) exposed to lethal radiation or *Sema4d*^{-/-} mice. Eight weeks after bone marrow transplant, a high level donor type chimerism (>95 %) was attained.

[0039]

[GeneChip Analysis]

We carried out GeneChip analysis in accordance with the method described in the literature (K. Nishikawa *et al.*, J Clin Invest 120, 3455 (Oct 1, 2010)). This means that after cDNA synthesis was carried out using reverse transcription using all of the RNA, we synthesized the cRNA which had been subjected to biotinized labeling by transcription *in vitro*. After we fragmented the cRNA, we carried out hybridization using the mouse A430 GeneChip (made by Affymetrix) in accordance with the method described in the literature (T. Koga *et al.*, Nat Med 11, 880 (Aug, 2005); H. Takayanagi *et al.*, Dev Cell 3, 889 (Dec, 2002).

[0040]

[Induction of Differentiation to Osteoblasts *in vitro*]

Induction of differentiation to osteoblasts and osteoclasts *in vitro* was carried out according to the method described in the document (T. Koga *et al.*, Nat Med 11, 880 (Aug, 2005); and H. Takayanagi *et al.*, Dev Cell 3, 889 (Dec, 2002). This means that we carried out differentiation induction by carrying out culturing of calvaria-derived cells in an osteogenesis culture medium (50 μ M of ascorbic acid, 10 nM of dexamethasone, and 10 mM of

β glycerophosphate) and the differentiation induction was confirmed by alkaline phosphatase (ALP) assay (after 7 days) and osteonodosity analysis (21 days later, alizarin red staining). Fc-sema4D, anti-Sema4D antibodies and anti-plexin BA antibodies (anti-Plexin-B1 antibodies) was added every 3 days. The osteoclast supernatant was recovered from each of the culture solutions of the wild type and Sema4d-/- cells after stimulating the intranuclear factor κ B activated receptor ligand (RANKL; made by Peprotech). We cultured the cultured osteoclasts in a collagen coated dish (made by IWAKI) and recovered it using trypsin two days after RANKL stimulation. The osteoclast supernatant was used as an osteogenesis culture medium containing the abovementioned reagent and we added it to the cultured osteoclasts (1 x 150 cell/well, 24 well-plate) every 3 days.

[0041]

[Quantitative Real-Time RT-PCR Analysis]

Quantitative real-time RT-PCR was carried out in accordance with the product protocols using a light cycler device (made by Roche) and SYBR Green (made by Toyobo). We used the following primers.

Plxnb1 sense: 5' -tggtcatgtgcagtacgat-3' (sequence number 5),
 Plxnb1 antisense: 5' -cactgctctccagggtctcc-3' (sequence number 6),
 Plxnb2 sense: 5' -aggggagcctctctacaagc-3' (sequence number 7),
 Plxnb2 antisense : 5' -tcgatcccttcctcctgaac-3' (sequence number 8),
 Plxnb3 sense: 5' -atatgctgagcgtgccttct-3' (sequence number 9),
 Plxnb3 antisense: 5' -tgctgttgagcaaatggag-3' (sequence number 10),
 CD72 sense: 5' -gccttctctgtcctgtctg-3' (sequence number 11),
 CD72 antisense: 5' -cctcctggaactgctgagac-3' (sequence number 12),
 Alpl sense: 5' -aaccagacacaagcattcc-3' (sequence number 13)
 Alpl antisense: 5' -gcctttgaggttttgggtca-3' (sequence number 14)
 Bglap sense: 5' -gcgctctgtctctctgacct-3' (sequence number 15),
 Bglap antisense: 5' -accttattgccctcctgctt-3' (sequence number 16),
 Colla1 sense: 5' -gagcggagagtactggatcg-3' (sequence number 17),
 Coll1l antisense: 5' -gttcgggctgatgtaccagt-3' (sequence number 18)
 Gapdh sense: 5' -accagaaagactgtggatgg-3' (sequence number 19),

[0042]

[Genetic Introduction of Adenovirus and Retrovirus]

The method of producing the adenovirus vector carrying the configuration active type (CA) of RhoA (Myc-V14Rho) and Rac1 (hRac1 V12) and the dominant negative type (DN) of the RhoA (Myc-N19Rho) and Rac1 (hRac1 V12) and the method of introducing these were carried out in accordance with the method described in the literature (Bito, H. *et al.* A Critical Role for a Rho-Associated Kinase, p160ROCK, in Determining Axon Outgrowth in Mammalian

CNS Neurons, Neuron 26, 431-441 (2000). Producing the retrovirus vector (pMXs-Plexin-B1-EGFP, pMXs-Plexin-B1 RA-EGFP and pMXs-Plexin-B1 DPDS-EGFP where the mutant [Plexin-B1 DPDZ-EGFP]) manifested wherein activation of [Plexin-B1 RA] and RhoA could not be activated was carried out by inserting the cDNA fragment of respectively Plexin-B1, Plexin-B1 RA (I. Oinuma, Y. Ishikawa, H. Katoh, M. Negishi, Science 305, 862 (Aug 6, 2004) and Plexin-B1 DPDZ (V. Perrot, J. Vazquez-Prado, J.S. Gutkind, J Biol Chem 277, 43115 (Nov 8, 2002)) in pMXs-IRES-EGFP. Production of a recombinant retrovirus was carried out in accordance with the method described in the literature (S. Morita, T. Kojima, T. Kitamura, Gene Ther 7, 1063 (Jun, 2000). This means that we carried out a retrovirus packaging by introducing the retrovirus vector produced in the Plat-E cells.

[0043]

[Anti-Sema4D Anti-Body Processing for OVX Induced Decline in Bone Quantity]

Producing osteoporosis model mice induced by OVX (ovaries extracted) was carried out in accordance with the method described in the literature (M. Shinohara *et al.*, J. Bio Chem 282, 17640 (Jun 15, 2007) . This means that we carried out an ovarian extraction operation or pseudo operation on 7 week old female mice. Of these model mice, we carried out tests on more than 6 mice in each group. We validated this using the method indicated below to find out whether or not there was a preventive effect on the decline in bone quantity. This means that we injected intravenously 20 μ g of Sema4D antibodies (made by MBL) or a saline solution from the caudal vein once a week. We sacrificed all of the mice 8 weeks after surgery and carried out μ CT analysis and osteomorphogenesis analysis. We also carried out validation using the following method to study whether or not there was an acceleration effect on the bone quantity which had decreased. This means that after 6 weeks we injected intravenously 20 μ g of anti-Sema4D antibodies (made by MBL) from the caudal vein for 3 weeks every 3 days in OVX mice. We sacrificed all of the mice 9 weeks after the operation and used them for μ CT analysis.

[0044]

[Immunoblotting Analysis, Pull Down Analysis and Immunofluorescent Staining]

We cultured calvarial cells for two days in an osteogenesis culture medium (50 μ M of ascorbic acid, 10 nM of dexamethasone and 10 mM of β -glycerophosphate) and then stimulated these with Fc-Sema4D. We used purified human IgG (Fc part) (made by Beckman Coulter) for native control for pull down analysis (time 0). We collected cells at the point indicated and carried out immunoblotting analysis or pull down analysis using anti-Plexin-B 1 antibodies (clone A-8, made by Santa Cruz); anti-PDZ-RhoGEF antibodies (polyclonal, made by Protein Express) ; anti-LARG antibodies (polyclonal, made by Lifespan Biosciences); anti-phospho-Akt antibodies (Thr308) (polyclonal, made by Cell Signaling); anti-Akt antibodies (polyclonal, made by Cell Signaling); anti-phosphor ERK antibodies (Thr202/Tyr204) (polyclonal, made by Cell Signaling); anti-ERK antibodies (polyclonal, made by Cell Signaling); anti-Met antibodies

(clone 25H2) (made by Cell Signaling); anti-ErbB2 antibodies (clone 29D8) (made by Cell Signaling); anti-Rac1 antibodies (clone 102/Rac1) (made by BD Transduction Laboratories); anti-RhoA antibodies (clone 55/Rho) (made by BD Transduction Laboratories); anti-cadherin-11 antibodies (polyclonal, made by Invitrogen); anti-IRS1 antibodies (clone 53-10C-31) (made by Millipore); and anti-b-actin antibodies (clone AC40) (made by Sigma-Aldrich). The phosphorylation of plexin B1, Met, ErbB2 and IRS1 was detected by anti-phosphotyrosine antibodies (4G10, made by Upstate) after immunoprecipitation using respectively specific antibodies for these. We incubated a cell dissolved product with Fc-sema4D (500 ng) which binded with protein A-agarose beads in order to detect the plexin B1 which binded to semaphorin 4D and carried out immunoblotting analysis using anti-Plexin-B1 antibodies. Detecting activation of the GTPase was carried out in accordance with the description in the literature (M. Shinohara *et al.*, J Bio Chem 282, 17640 (Jun 15, 2007). This means that we treated the calvarial cells with Fc-sema4D and collected them at the point they were displayed. We incubated the cell dissolved product with GST-RBD (using RhoA) or GST-PAK1 (using Rac1) (2 μ g) which binded with glutathione sepharose and we carried out immunoblotting analysis using respectively anti-RhoA antibodies or anti-Rac1 antibodies. We fixed the cells with a 4 % paraform aldehyde for immunofluorescent staining, carried out permeation processing and then stained it using Alex Fluor 488 labeling secondary antibodies and rhodamine conjugate phalloidin (made by Molecular Probes).

[0045]

[Flow Cytometry]

We stained a single cell floating solution using monoclonal antibodies conjugated with 8 types of fluorescent pigments [anti-CD45. 2 antibodies conjugated with PerCP.Cy5.5; anti-CD45.2 antibodies (clone 104) conjugated with FITC; anti-CD11b antibodies (clone M1/70) conjugated with eFLuor450; anti-CD105 antibodies (clone MJ7/18) conjugated with PE; anti-CD10 antibodies (clone 4.29E+02) conjugated with Alex Fluor 647; (anti-CD44 antibodies conjugated with APC-Alexa Fluor 750; anti-Cd 44 antibodies (clone IM7) conjugated with AC-Cy7; and anti-Sca-1 antibodies (clone D7) (made by eBioscience) conjugated with APC in order to analyze the bone marrow derived cells and the calvarial cells. Then, we carried out flow cytometry using FACSCant II using Diva software (made by BD Biosciences).

[0046]

[Cell Proliferation Analysis]

We cultured bone marrow-derived interstitial cells in an osteogenesis condition culture medium and analyzed the cell proliferation rate before osteoblast differentiation (Day 0) or in the osteoblast differentiation process (Day 14) using semaphorin4D stimulation using human IgG Fc partial or using cell proliferation ELISA kit (made by Roche) in the presence of Fc-Semar4D and detected the incorporation of 5-bromo-2' deoxyuridine (BrdU).

[0047]

[Analysis of Colony Formation Unit (CFU)]

We plated the bone marrow cells at 3×10^6 cells per single well on a 24-well plate. Then, we cultured this for 3 days using α -MEM culture medium containing 10 % fetal calf serum and then replaced it with an osteogenesis condition culture medium. The colony formation unit-alkali phosphatase (CFU-ALP) was detected as an ALP positive colony on the 7th day and the CFU-osteoblast (CFU-Ob) was detected as an alizarin red positive colony on day 21. The aggregate number of colonies (CFU-fibroblasts (CFU-F)) was detected by staining with toluidine blue on day 7 and day 21.

[0048]

[Statistical Analysis]

All of the data are indicated as mean $\pm$ SEM (n=15). Statistical analysis was carried out using the Student's test A NOVA and when possible the Bonferroni test (*p < 0.05; ** p<0.01; *** p<0.005; n.s., not significant). Results are indicated in the representative example of 4 or more individual experiments.

[0049]

[Results]

We carried out mRNA genome wide screening in the osteoclasts and the osteoblasts using GeneChip analysis to study whether or not the semaphorin, ephrin, slits and netrin which are axon guidance molecules contribute to the remodeling of the bone. This means that after analyzing 20 kinds of semaphorin, 16 types of ephrin, 6 types of slits and 6 kinds of netrin, an extremely high manifestation of semaphorin 4D was confirmed in the osteoclasts. Meanwhile, this manifestation was not confirmed in the osteoblasts. These results indicate that the semaphorin 4D had a selectively induced manifestation in the osteoclast formation process.

[0050]

We carried out a functional analysis of the skeletal system using Sema4d^{-/-} mice to study the semaphorin 4D function with selectively induced manifestation in the osteoclast formation process. The bone quantity (Figure 1) and the trabecular width (Figure 2) in the Sema4d^{-/-} mice was shown to have increased significantly compared to the wild-type mice (WT mice) by using μ CT and osteomorphogenesis analysis. The surface area of the osteogenesis in Sema4d^{-/-} mice (Figure 3, at left), the surface area of the calcified bone (Figure 3, middle) and the osteogenesis rate (Figure 3, at right) increased conspicuously as compared to the wild type mice. However, there were no changes in the parameters (surface areas of osteoclasts (Figure 4, at left), the number of osteoclasts (Figure 4, center) and the surface area of the corroded bone (Figure 4, at right) indicating the bone resorption of the osteoclasts. We also observed that the *in vitro* osteoclast formation in Sema4d^{-/-} cells was normal. Despite the fact that the semaphorin 4D

manifested specifically in the osteoclasts, these results suggest that the phenotype of high bone density in the *Sema4d*^{-/-} mice increased due to osteogenesis through the osteoblasts.

[0051]

We carried out adoptive immune cell grafting using bone marrow cells which included osteoclast precursor cells to find out whether or not the *Sema4d*^{-/-} mice bone phenotype was based on abnormalities in the bone marrow cell system. As a result, the bone quantity increased when the *Sema4d* deficient bone marrow cells were grafted onto the wild mice as compared to when wild type bone marrow cells were grafted (see the two graphs on the left in Figure 5). Meanwhile, when wild type bone marrow cells were grafted onto *Sema4d*^{-/-} mice, the bone quantity decreased and returned to normal as compared to when the *Sema4d* deficient bone marrow cells were grafted (see the two graphs on the right in Figure 5). Based on these results, it was indicated that the bone phenotype in the *Sema4d*^{-/-} mice was such that abnormalities in the hematopoietic system cells comprising the osteoclasts were the cause. Furthermore, no clear-cut increase in the osteonodes of the *Sema4d*^{-/-} calvarial cells was confirmed in the osteonodosity of the *Sema4d*^{-/-} calvarial cells. These results suggest that the semaphorin 4D which manifests in the osteoclasts functions as a bone remodeling factor inhibiting osteogenesis through the osteoblasts.

[0052]

We produced Fc-sema4D (I. Ishida *et al.*, Int Immunol 15, 1027 (Aug, 2003) to study in detail the inhibition effect of the semaphorin 4D on the osteoblasts. Then, we added the Fc-sema4D to the calvarial cells which had been cultured under osteogenesis conditions. Adding the Fc-sema4D inhibited concentration dependently activation of ALP and manifestation of osteocalcin [Bglap] and collagen I type [Colla1]). These results indicate that the osteonodosity is inhibited as a result of the Sema4D inducing differentiation of the osteoblasts. We also cultured the calvarial cells in the presence of the culturing supernatant of the osteoclasts or in the presence of the osteoblasts to see whether or not the semaphorin 4D derived from the osteoclasts contributed to the regulation of the osteogenesis. In joint culturing with the culture supernatant of the wild-type osteoclasts or with the osteoclasts, there was no effect on the osteonodosity whereas in joint culturing with the culture supernatant of the *Sema4d*^{-/-} osteoclasts or in joint culturing with the osteoclasts, osteonodosity was clearly accelerated. Based on these results, the osteoclasts are indicated as inhibiting osteogenesis through the semaphorin 4D produced as soluble at least partially. It must also be pointed out that one or more factors are contained in the supernatant of the osteoclasts. The osteogenesis acceleration effect brought about by these factors is thought to be brought out to the fullest when semaphorin 4D is not present.

[0053]

We analyzed (a) the manifestation of the plexin B type which is well known as a semaphorin D receptor in non-lymphatic cells and lymph cells and (b) the RNA of CD72 (K.

Suzuki, A. Kumanogoh, H. Kikutani, *Nat Immunol* 9, 17 (Jan, 2008) using quantitative real time RT-PCR in order to specify the semaphorin 4D receptor where the osteoblasts manifested. As a result, the amount of plexin B1 manifested increased conspicuously during osteoblast differentiation (Figure 7). Meanwhile, the amount of plexin B2 manifested was rather low and there was virtually no manifestation plexin B 3 and CD 72 (Figure 7). After carrying out pull down analysis using Fc-sema 4D, it was indicated that the semaphorin 4D interacted with plexin B1 (Figure 8). It was also indicated that Fc-sema 4D induces phosphorylation of plexin B 1. It is well known that when semaphorin 4D binds with plexin B1, a conjugate is formed with tyrosine kinase ErbB2 and ErbB2 phosphorylates itself and plexin B1 is dependent on semaphorin B4. In osteogenesis, after inhibiting ErbB2 kinase activation, the phosphorylation declines dependent on the semaphorin 4D stimulation of plexin B1. Based on these results, it is suggested that the plexin B1 acts as the main receptor for the semaphorin 4D in the osteoblasts. Then, we analyzed the bone phenotype of the *Plxb1*^{-/-} mice. Like the *Sema4d*^{-/-} mice, the bone quantity (Figure 9) and the width of the trabecula (Figure 10) of the *Plxb1*^{-/-} mice increase conspicuously as compared to the wild type mice due to an increase in the osteoblast osteogenesis. The surface area of the osteoblasts in the *Plxb1*^{-/-} mice (Figure 11), the surface area of the calcified bone (Figure 12) and the osteogenesis rate (Figure 13) also increased respectively as compared to the wild-type mice. However, there was no change in the parameters (surface area of the osteoclasts, osteoclast cell count and corrosion of bone surface area) indicating the bone resorption in the osteoclasts. Based on these results, it is suggested that the phenotype of high bone density of the *Plxb1*^{-/-} mice, like the phenotype of the high bone density of the *Sema4d*^{-/-} mice, is caused by an increase in osteogenesis brought about by the osteoblasts. No stimulation effect on the osteonodosity by the osteoblasts confirmed by the wild-type *Sema4d*^{-/-} osteoclasts was confirmed with the *Sema4d*^{-/-} osteoclasts. Therefore, this suggests that plexin B1 recognizes the osteogenesis mainly through semaphorin 4D.

[0054]

How does the semaphorin 4D-plexin B1 transform to the inhibition signals in the osteoblasts? It is well known that the semaphorin-plexin system regulates osteomorphogenesis and cell wandering by regulating the rearrangement of the actin cell bone. It is also well known that when semaphorin 4D is combined with plexin B2, the RhoA activity is stimulated in the presence of tyrosine kinase ErbB2 whereas the semaphorin 4D has a counter action in the presence of other tyrosine kinase Met (J.M. Swiercz, T. Worzfeld, S. Offermanns, *J Bio Chem* 283, 1893 (Jan 25, 2008). Therefore, we studied the possibility of semaphorin 4D activating the RhoA. When we analyzed the amount of ErbB2 and Met manifested in the osteogenesis, it was suggested that ErbB2 manifested in considerably greater quantities than the Met. Although semaphorin 4D stimulation induced phosphorylation of the ErbB2, phosphorylation of Met was not induced. Furthermore, based on immunoblotting analysis and pull down analysis, semaphorin 4D increased the GTP combining activation type for RhoA and it is indicated that this activity is inhibited particularly by the *Plxb1*^{-/-} cells. Meanwhile, the activity of Rac1

which is in another Rho family did not affect the semaphorin 4D stimulation. Coinciding with these results, when we introduced structured activation type RhoA using an adenovirus, the osteonodosity in the calvarial cells was inhibited whereas when dominant negative RhoA was introduced, the osteonodosity was accelerated. Meanwhile, the structured activation type Rac1 and the dominant negative Rac1 did not affect the osteogenesis. Based on these results, it is suggested that the RhoA selectively mediates the inhibition effect for the osteogenesis of semaphorin 4D · plexin B1. Plexin B1 has two RhoGTPase regulation domains, that is, a GTPase activated protein (GAP) domain and a PDZ-binding domain which binds to Rho-GEF (I. Oinuma, Y. Ishikawa, H. Katoh, M. Negishi, *Science* 305, 862 (Aug 6, 2004), J.M. Swiercz, R. Kuner, J. Behrens, S. Offermanns, *Neuron* 35, 51 (July 3, 2002), V. Perrot, J. Vazquez-Prado, J.S. Gutkind, *J Bio Chem* 277, 43115 (Nov 8, 2002), M.H. Driessens, C. Olivo, K. Nagata, M. Inagaki, J.G. Collard, *FEBS Lett* 529, 168 (Oct 9, 2002). Based on pull down analysis and immunoblotting analysis, PDZ-RhoGEF and LARG (leukemia associated RhoGEF) which are known as Rho-GEF were indicated as binding with plexin B1 even in the osteoblasts. Therefore, the abovementioned GAP domain and PDZ-binding domain produced two types of plexin B1 mutants (Plexin B1-Δ PDZ and Plexin B1 · RA) to determine whether the osteoporosis was significantly inhibited (I. Oinuma, Y. Ishikawa, H. Katoh, M. Negishi, *Science* 305, 862 (Aug 6, 2004) (Figure 14). After the two types of plexin B1 mutants and wild type plexin B1 (WT-Plexin · B1) were used respectively to produce Plxnbl^{-/-} calvarial cells with excess manifestation, we stimulated these using Fc-sema 4D and evaluated the osteoblast differentiation inhibition effect. The inhibition effect of the Fc-Sema4d indicated in the manifestation of mRNA of the osteoblast markers (Alpl, Bglap and Colla1) was recovered in the WT-Plexin B1 and PlexinB1-RA excess manifestation, however, it was not recovered in the PlexinB1-Δ PDZ excess manifestation. These results suggest that RhoA mediates the inhibition of osteogenesis specifically by the semaphorin 4D.

[0055]

We crossed CAT-RHoA DN transgenic mice (K. Kobayashi *et al.*, *J. Neurosci* 24, 3480 (Apr 7, 2004) and collagen α 1 (I)-Cre transgenic mice (R. Dacquin, M. Starbuck, T. Schinke, G. Karsenty, *Dev. Dyn* 224, 245 (Jun, 2002)) and produced mice in which dominant negative RhoA was manifested specifically (RhoA DNOB) in the osteoblasts to study the role of RhoA in the osteoblasts *in vivo*. The bone quantity (Figure 15) and the width of the trabecula (Figure 16) of the RhoA DNOB mice increased as compared to the wild type mice when osteogenesis was accelerated by the osteoblasts. In addition, the surface area of the osteoblasts (Figure 17) of RhoA DNOB mice, the surface area of the bone (Figure 18) which had calcified and the osteogenesis rate (Figure 19) increased conspicuously compared to the wild-type mice, however, there were no changes in the parameters which indicated the bone resorption of the osteoclasts (surface area of osteoclasts, number of osteoclasts and surface area of corroded bone). The bone phenotype was the same as the bone phenotype for the Sema4d^{-/-} and Plxbl^{-/-}-mice. As the manifestation of the RhoA DNOB increased during osteoblast differentiation, the manifestation

of the osteonodosity and osteoblast marker genes (Alpa, Bglap and Colla1) increased markedly in the calvarial cells originating in the RhoA DNOB mice. The osteonodosity which increased in the RhoA DNOB cells was not inhibited by the Fc-sema4d so that it is suggested that the RhoA mediates the inhibition signals downstream of the semaphorin 4D-plexin B1.

[0056]

In order to study whether or not the Sema4D function inhibition was effective for osteoporosis, we carried out validation using model mice with osteoporosis after menopause treated for OVX (ovaries excised). This means that we administered intravenously the anti-Sema4D antibodies in mice on a weekly basis following OVX treatment and studied whether there was a preventive effect for a decrease in bone quantity. After analyzing the bone tissue, the bone quantity decreased when anti-Sema4d antibodies were not administered whereas when anti-Sema4d antibodies were administered, acceleration in osteogenesis (increase in surface area of osteoblast [Figure 20], bone quantity [Figure 21] and osteogenesis rate [Figure 22] and decrease in trabecular intervals [Figure 23]) was confirmed. Meanwhile, there were no changes in the parameters indicating bone resorption of the osteoclasts (number of osteoclasts and surface area of corroded bone). Based on these results, when the function of semaphorin 4D is inhibited by anti-Sema 4D antibody treatment, a preventive effect on the decrease in bone quantity in osteoporosis is indicated. We also studied whether or not the functional inhibition of semaphorin 4D was effective even for treatment of bone quantity which had already decreased. This means that after OVX treatment, we administered the anti-Sema4d antibodies from the caudal vein for three weeks three times a week 6 weeks after the bone quantity had decreased. The results of analyzing the parameters indicating osteogenesis (surface area of osteoblasts, increase in bone quantity, increase in osteogenesis rate and decrease in trabecular intervals) confirmed acceleration in osteogenesis (increase in surface area of osteoblast [Figure 26], bone quantity [Figure 27] and osteogenesis rate [Figure 28] and decrease in trabecular intervals [Figure 29]). Therefore, it became clear that once the bone quantity had decreased it returned to the same level as when Sema4D antibodies were administered on a weekly basis after the abovementioned OVX treatment. This means there was a return to the same level as when decrease in bone quantity was prevented. Meanwhile, there were no changes in the parameters indicating bone resorption of the osteoclasts (surface area of corroded bone). We also studied the effect of the functional inhibition of semaphorin 4D and plexin B1 on osteonodosity. When mouse osteoblasts and the osteoclasts of WT mice were used, osteonodosity was accelerated as a function of the concentration of anti-Sema 4D antibodies (plates 1 to 3 from the left in the upper panel of Figure 24). Meanwhile, the osteonodosity which was inhibited by Fc-sema4d was accelerated as a function of the concentration of anti-plexin B1 antibodies (lower panel in Figure 24). Based on these results, when the semaphorin 4D-plexin B1 interaction is inhibited by anti-Sema 4d antibody treatment or anti-plexin B1 antibody treatment, not only is the decrease in bone quantity in osteoporosis inhibited but an effect which promotes an increase in bone quantity is indicated as well. We also studied the effect of the functional inhibition of semaphorin 4D in

human osteoblasts on osteonodosity. This means that when we carried out validation using osteoclasts which had differentiated from the human peripheral blood monocyte derived CD14 positive cells (Figure 25, lower row) and the culturing supernatant thereof (Figure 25, upper row) and when we added it to the osteoclasts and the culturing supernatant thereof and added anti-Sema4d antibodies to the osteoblasts, the osteonodosity was accelerated as a function of the concentration of the anti-Sema 4d antibodies (Figure 25). These results indicate that the results in the abovementioned post-menopausal osteoporosis model mice were supported in human cells and that inhibition of the semaphorin 4D-plexin B1 interaction is a new strategy in promoting osteogenesis.

[0057]

We made a detailed analysis of the action of the semaphorin RD osteoblast differentiation inhibition. This means that when we compared the number of hematopoietic cells in the bone marrow (Sca-1+CD105+CD106+CD44+CD45, 2-CD11b) in the Sema4d-/-mice with the wild-type mice by carrying out flow cytometry analysis, there were no differences. We also carried out cell proliferation analysis and although there was somewhat of an increase due to the Sema4d prior to the differentiation of the osteoblasts (Day 0), there were no changes due to the semaphorin 4D stimulation in the osteoblast differentiation process (Day 14). Furthermore, based on CFU analysis, the osteogenesis indicated in the ALP manifestation and the alizarin was inhibited due to the semaphorin 4D stimulation. These results suggest that the semaphorin 4D acts in osteoblast differentiation stages.

[0058]

Insulin receptor substrate (IRS) signals caused by insulin-like growth factors (IGF)-1 are known to accelerate the differentiation of the osteoblasts. Therefore, we studied Akt and ERK phosphorylation relating to the IRS signals with semaphorin 4D stimulation and the phosphorylation level declined. The Tyr phosphorylation which is the IRS activation indicator also declined. These results suggest that semaphorin 4D stimulation decreases the IRS signals. It is also clear that the activated type RhoA lowers the Akt and ERK phosphorylation and conversely Y-27632 and RKI which are RhoA inhibitors promote this phosphorylation. It is also clear that the RhoA inhibitor induces the promotion of activation type phosphorylation in the IRS signals. These results suggest that semaphorin 4D induces a decline in IRS signals by activating the RhoA and inhibits osteoblast differentiation.

[0059]

[Summary]

Based on the abovementioned experiments, we identified osteoclast-derived semaphorin 4D as an extremely important mediator for transmission between osteoclasts-osteoblasts in bone reconstruction. Bone reconstruction is carried out in cycles made up of three stages (beginning of bone resorption due to osteoclasts, transition to new osteogenesis due to osteoblasts and

completion of new bone synthesis (K. Matsuo, N. Irie, Arch Biochem Biophys 473, 201 (May 2008). Semar4d which manifests in osteoclasts functions up to the time osteoclast resorption is completed as an osteogenesis inhibition factor in the initial stage in which differentiation of the osteoblasts is inhibited. Furthermore, activation of RhoA is also indicated as inhibiting osteoblast osteogenesis *in vivo*. EphrinB2/EphB4 indicated as contributing to the transition stage also make use of RhoA to regulate the osteogenesis (C. Zhao *et al.*, Cell Metab 4, 111 (Aug, 2006) which suggests that the Rho family-lower molecular GTPase acts as a coordinator for bone reconstruction single transmission. The PDZ-containing RhoA specific GEFArfgef 12 (LARG) (J.M. Swiercz, R. Kuner, J. Behrens, S. Offermanns, Neuron 35, 51 (Jul 3, 2002); V. Perrot, J. Vazquez-Prado, J.S. Gutkind, J Bio Chem 277, 43115 (Nov 8, 2002)) has been indicated to have high manifestation in osteoblasts in GeneChip analysis. These results back up the importance of the semaphorin 4D-plexin B1-RhoA path. Furthermore, based on analysis using immunofluorescent staining, a down regulation for cadherin 11 has been observed after Fc-ema Rd stimulation and the contribution of the regulated gap binding function is suggested. Intermittent parathyroid hormones (PTH) treatment is the only effective method which has been proved to increase osteogenesis. Anti-Sost antibodies which are currently in development have garnered attention as a novel osteogenesis agent while antibodies targeting factors related to the semaphorin 4D-plexin B1-RhoA path and inhibitors and the like can be expected to be new therapeutic agents for patients with osteopenia.

[Industrial Applicability]

[0060]

The present invention can be used to effect in accelerating osteogenesis and in the prevention and treatment of bone diseases.

[Sequence Table]

Patent Application P11-0052011-108642_0.app

CLAIMS

1. A method for the prevention and/or treatment of bone diseases comprising administering to a subject in need thereof a therapeutically effective amount of an agent comprising as an active ingredient a substance which inhibits binding of semaphorin 4D and plexin B1.
2. A method according to Claim 1 wherein the substance is an anti-semaphorin 4D antibody or functional fragment thereof.
3. A method according to Claim 1 wherein the substance is an anti-plexin B1 antibody or functional fragment thereof, or a protein containing plexin B1 extracellular regions.
4. The method according to claim 2 or claim 3, wherein the antibody or fragment thereof is monoclonal.
5. The method of any one of claims 2 to 4, wherein the functional fragment of the antibody is a F(ab')₂ fragment, a Fab' fragment, a Fab fragment, a Fv fragment, a disulphide-linked FV fragment, a single-chain FV (scFV) fragment, or a polymer of one or more of said fragments.
6. The method of any one of claims 2 to 5, wherein the antibody or fragment thereof is a human antibody or functional fragment thereof.
7. A method according to any one of Claims 1 to 6, wherein the bone diseases are selected from bone fracture, bone deficiency, osteoporosis, osteomalacia, osteopenia, lumbar pain, Paget's disease of bone, tonic myelitis, articular rheumatism and deformative arthrosis.
8. The method according to any one of claims 1 to 4, wherein the agent can accelerate osteogenesis and promote differentiation of osteoblasts.
9. The method of any one of claims 1 to 8, wherein the subject is a mammal.
10. The method of claim 9, wherein the subject is a human.
11. The method of any one of claims 1 to 10, wherein the agent can be administered orally, by vascular administration, by intravenous administration, by muscular administration, by

hypodermic administration, by transdermal administration, by nasal administration, by transpulmonary administration, or by any combination thereof.

12. A method for determining a candidate active ingredient for an osteogenesis acceleration agent comprising determining whether a substance being studied inhibits binding between semaphorin 4D and plexin B1, wherein if the substance inhibits said binding, the substance is determined to be a candidate active ingredient for an osteogenesis acceleration agent.

13. A method according to Claim 12 wherein the method used to determine whether the substance inhibits binding between semaphorin 4D and plexin B1 consists of the following steps (A) through (D):

- (A) a step wherein semaphorin 4D and plexin B1 are brought in contact with one another in the presence of the substance being studied;
- (B) a step wherein the degree of binding between semaphorin 4D and plexin B1 is measured;
- (C) a step wherein the degree measured in step (B) above is compared with the degree when not in the presence of the substance being studied;
- (D) a step wherein the substance being studied is determined to be a substance which inhibits binding between semaphorin 4D and plexin B1 when the degree measured in step (B) above is lower than the degree when not in the presence of the substance being studied.

14. A method of screening for a candidate active ingredient for an osteogenesis acceleration agent wherein the method according to Claim 12 or 13 is used to search substances being studied for a candidate active ingredient for an osteogenesis acceleration agent.

15. Use of a substance which inhibits binding of semaphorin 4D and plexin B1 in the manufacture of a medicament for the prevention and/or treatment of bone diseases.

16. The use according to Claim 15 wherein the substance is an anti-semaphorin 4D antibody or functional fragment thereof.

17. The use according to Claim 15 wherein the substance is an anti-plexin B1 antibody or functional fragment thereof, or a protein containing plexin B1 extracellular regions.

18. The use according to any one of Claims 15 to 17 wherein the bone diseases are selected from bone fracture, bone deficiency, osteoporosis, osteomalacia, osteopenia, lumbar pain, Paget's disease of bone, tonic myelitis, articular rheumatism and deformative arthrosis.

National University Corporation Tokyo Medical and Dental University
Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

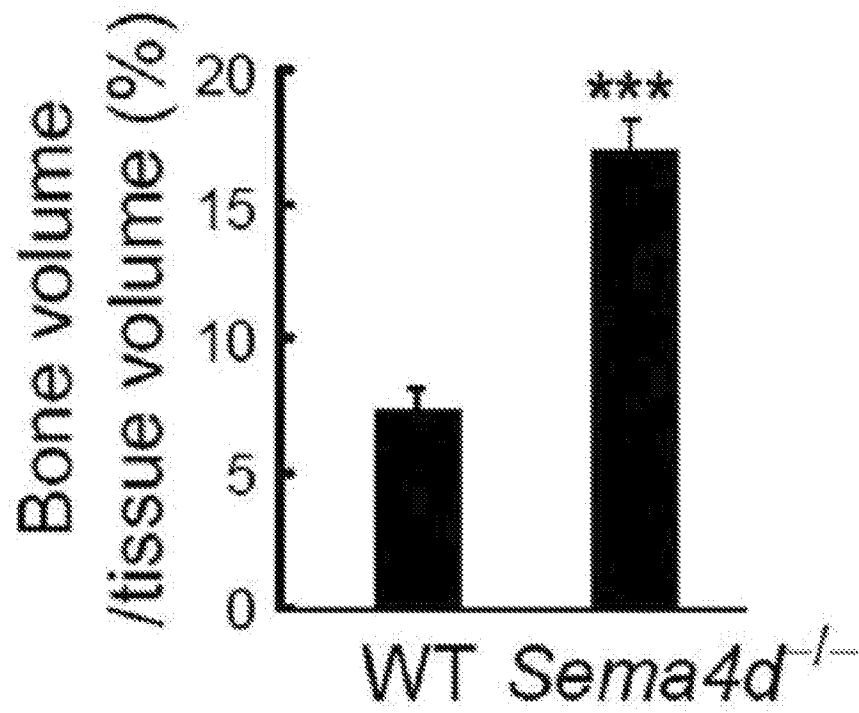


FIGURE 1

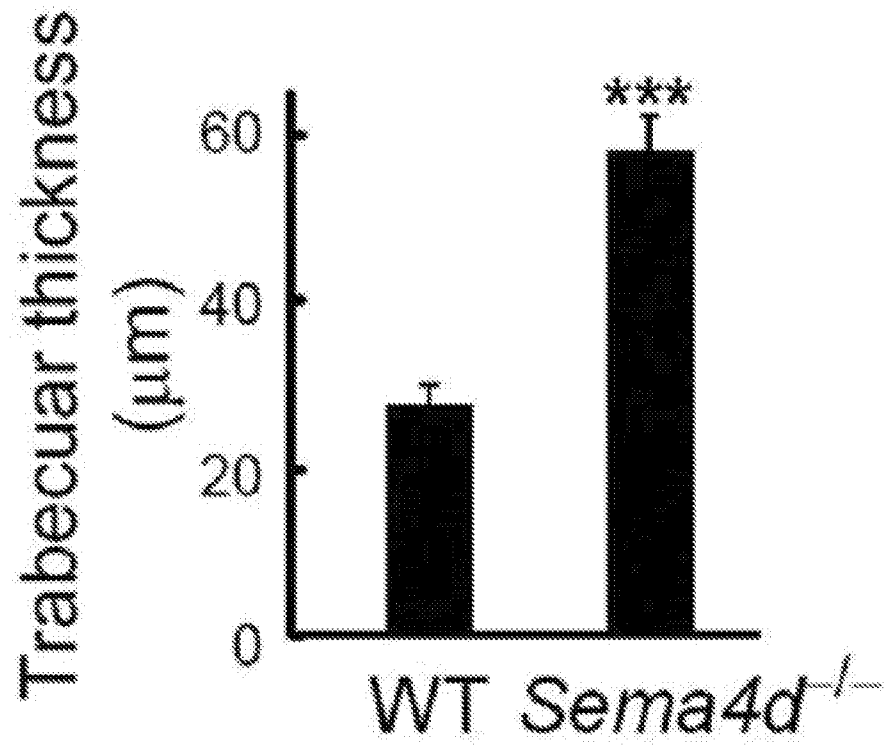


FIGURE 2

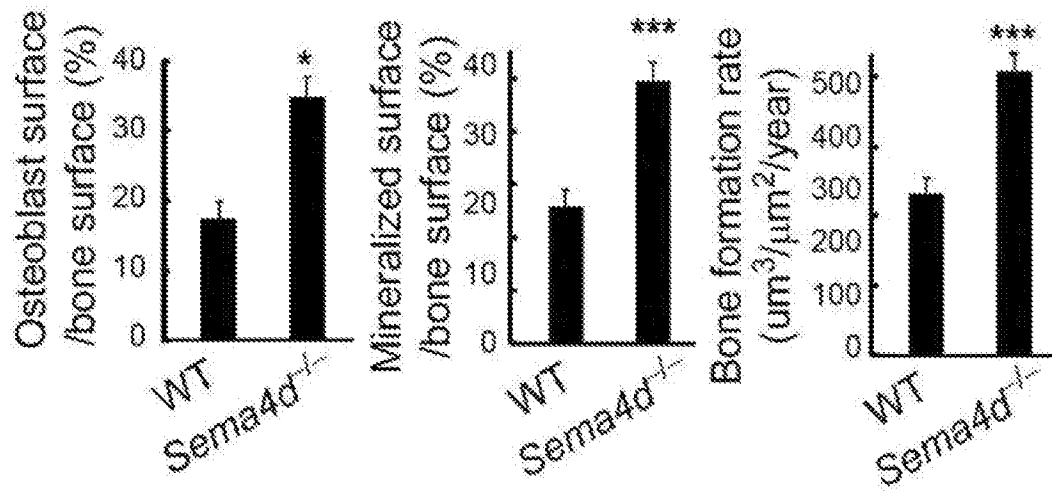


FIGURE 3

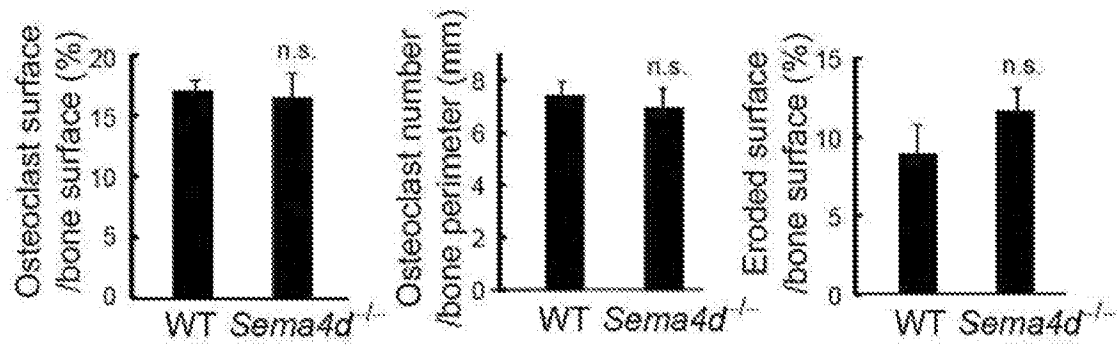


FIGURE 4

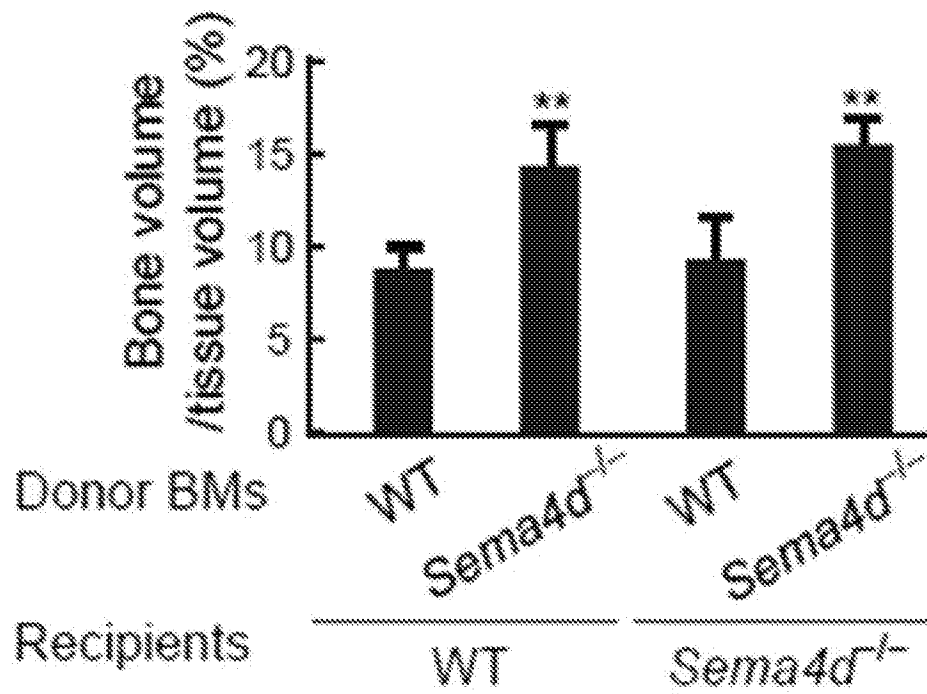


FIGURE 5

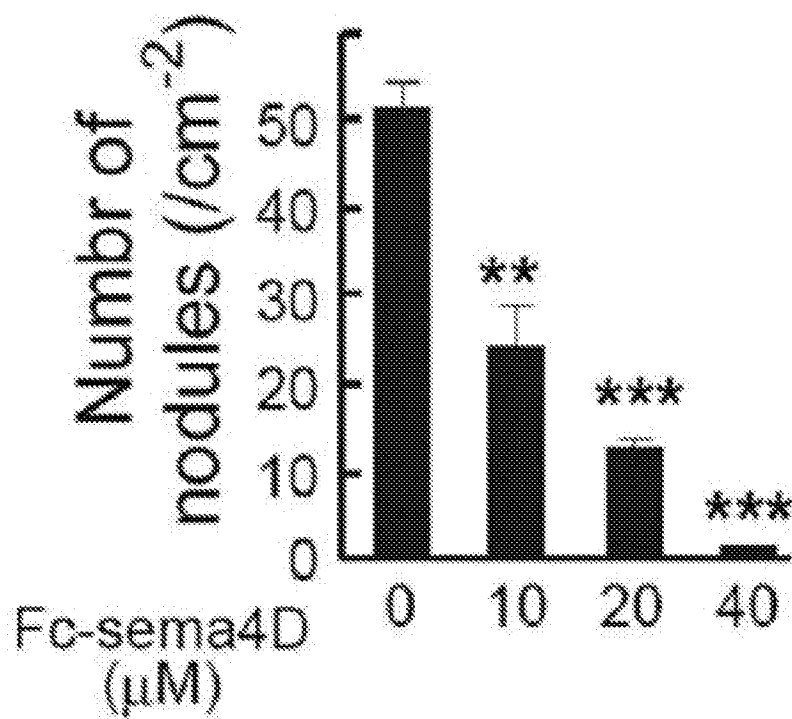


FIGURE 6

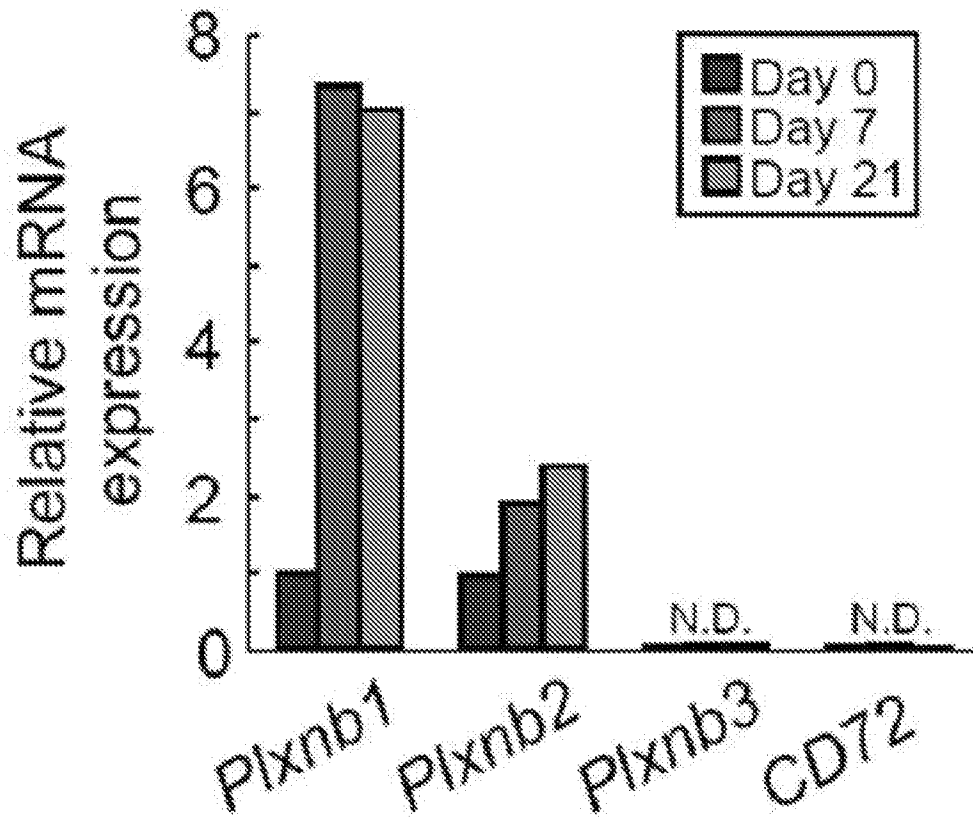


FIGURE 7

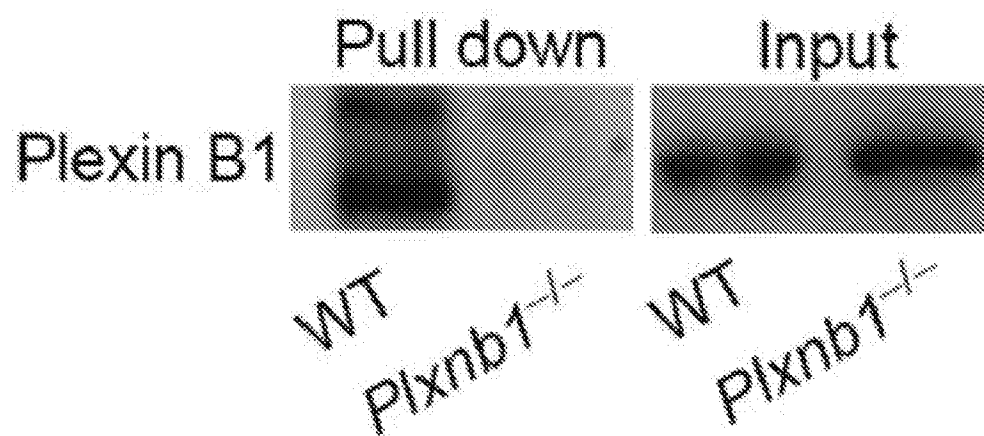


FIGURE 8

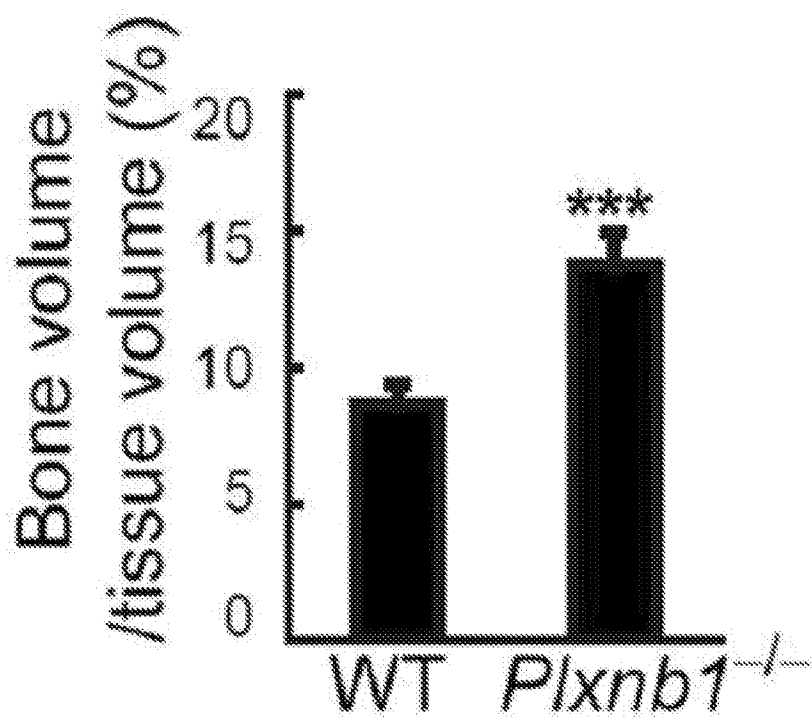


FIGURE 9

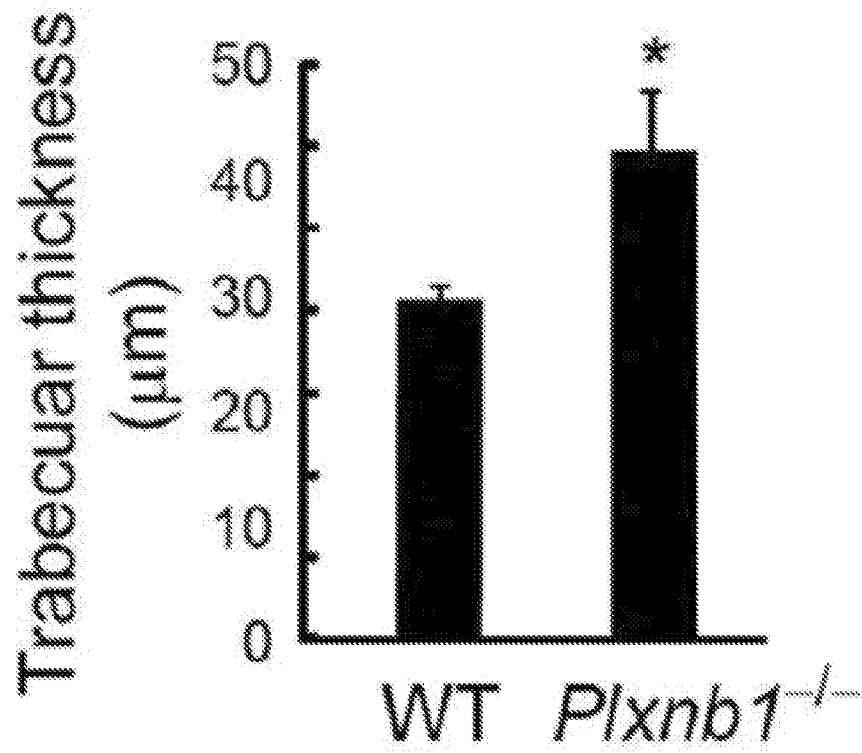


FIGURE 10

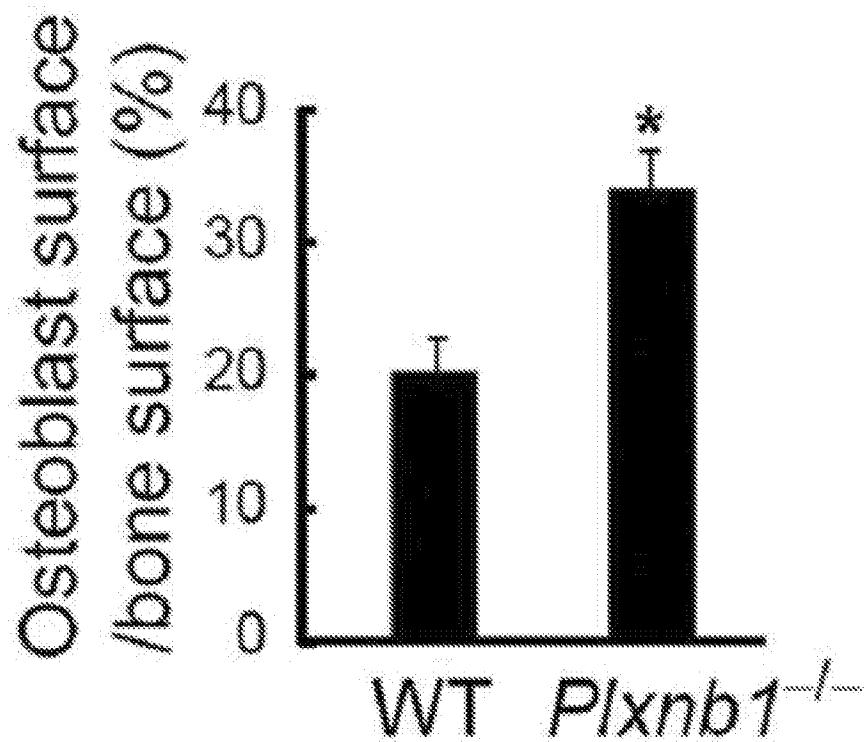


FIGURE 11

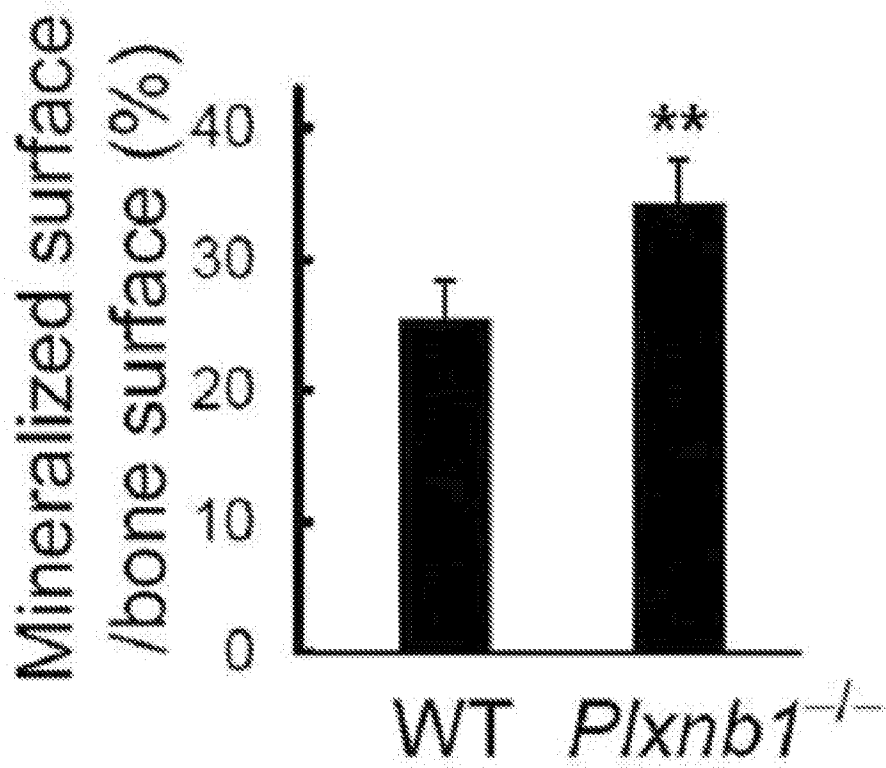


FIGURE 12

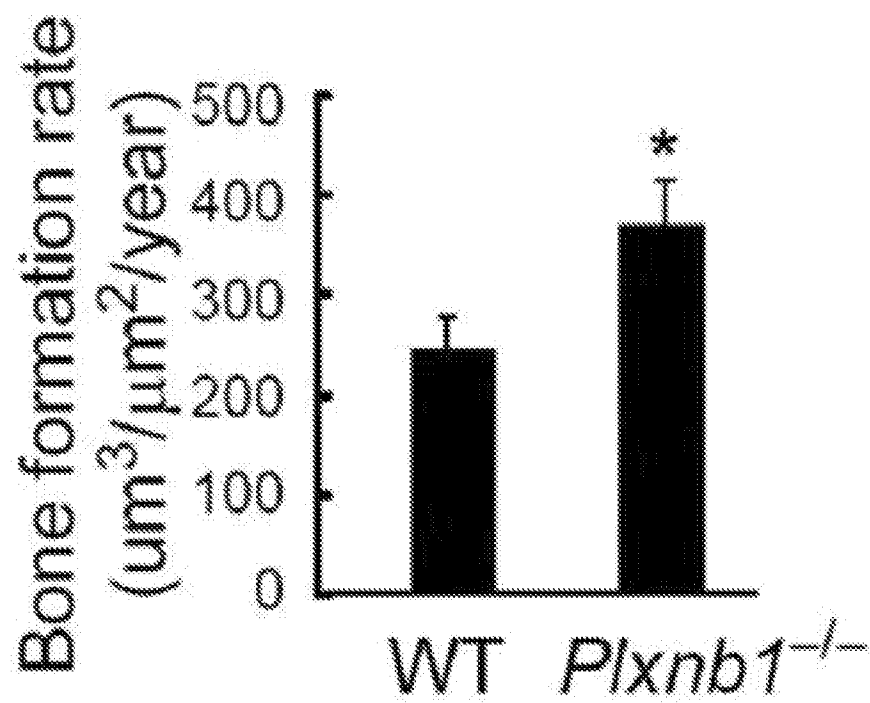
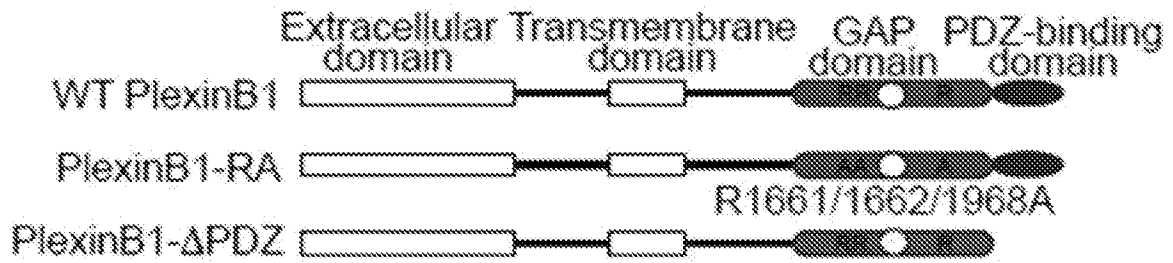


FIGURE 13

**FIGURE 14**

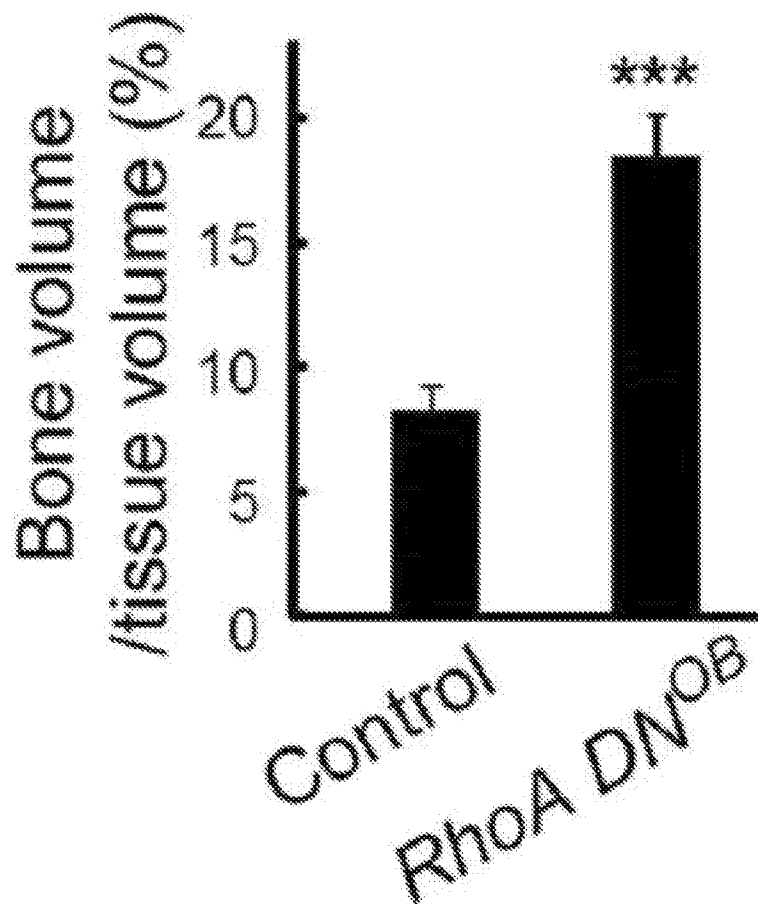


FIGURE 15

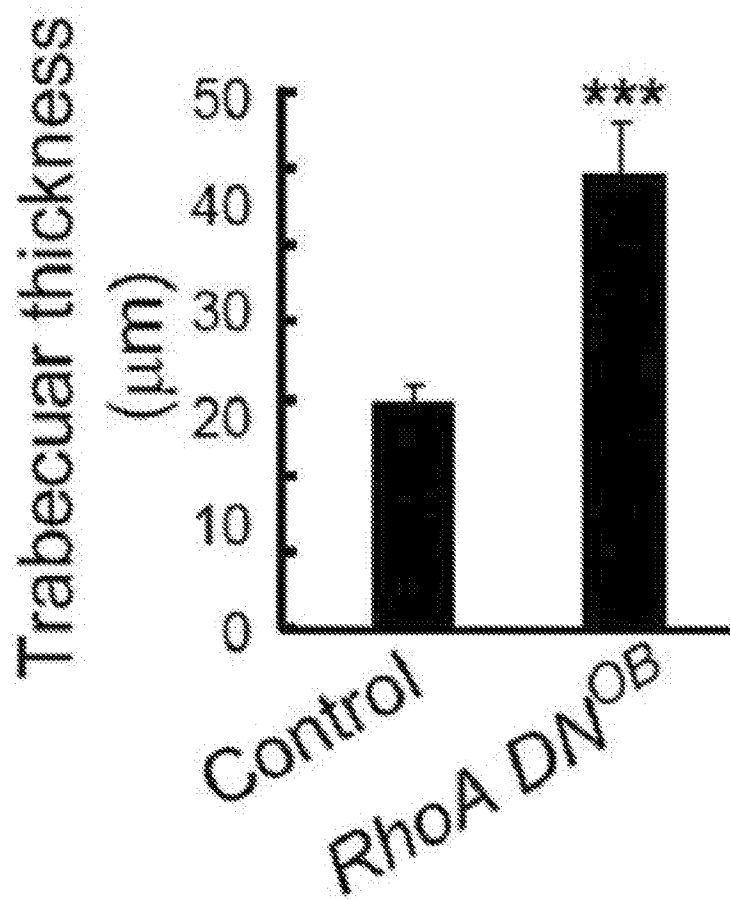


FIGURE 16

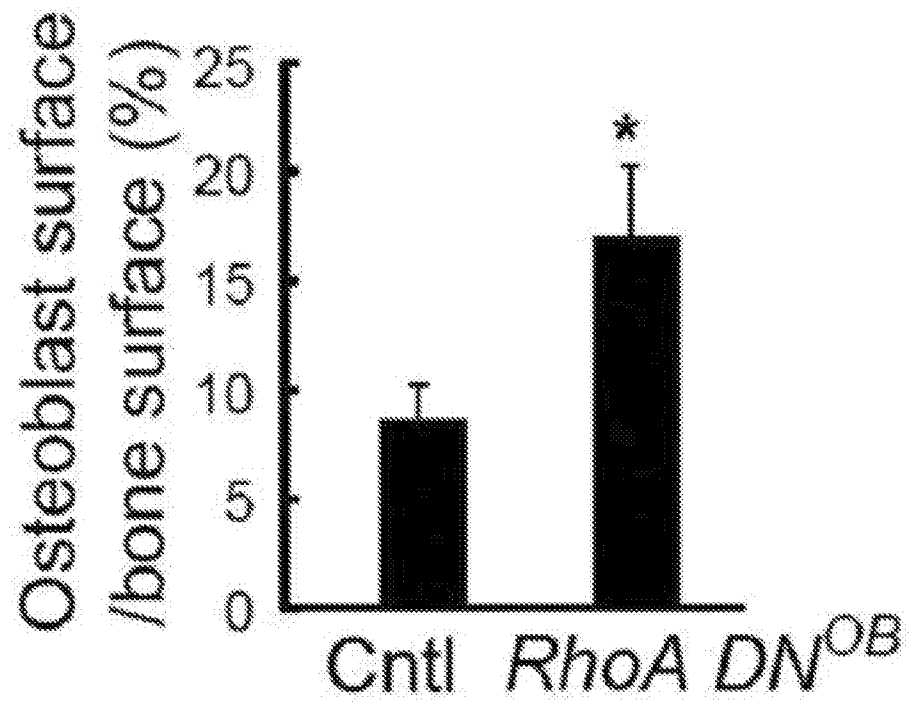


FIGURE 17

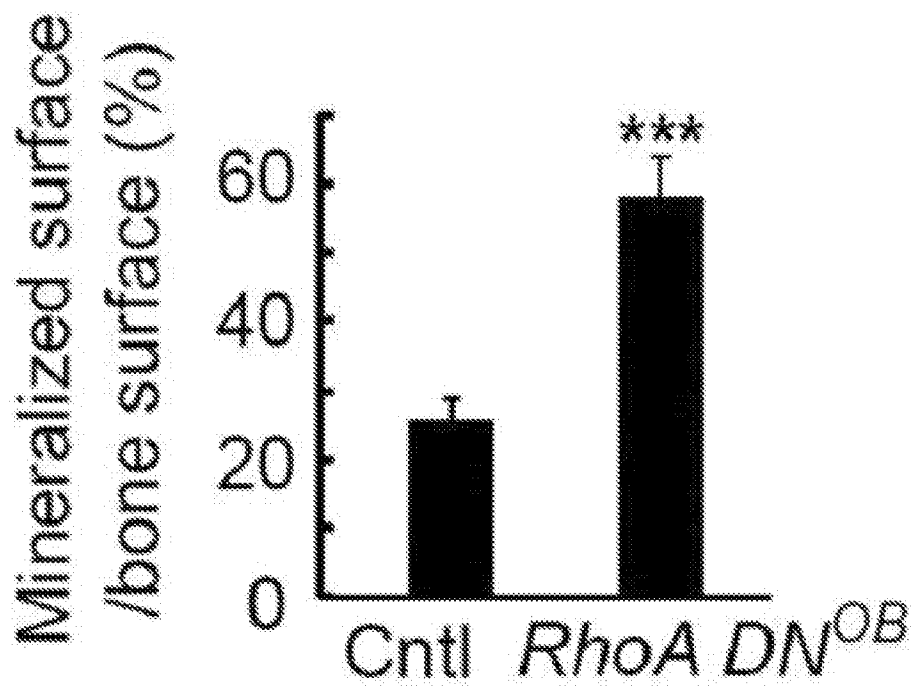


FIGURE 18

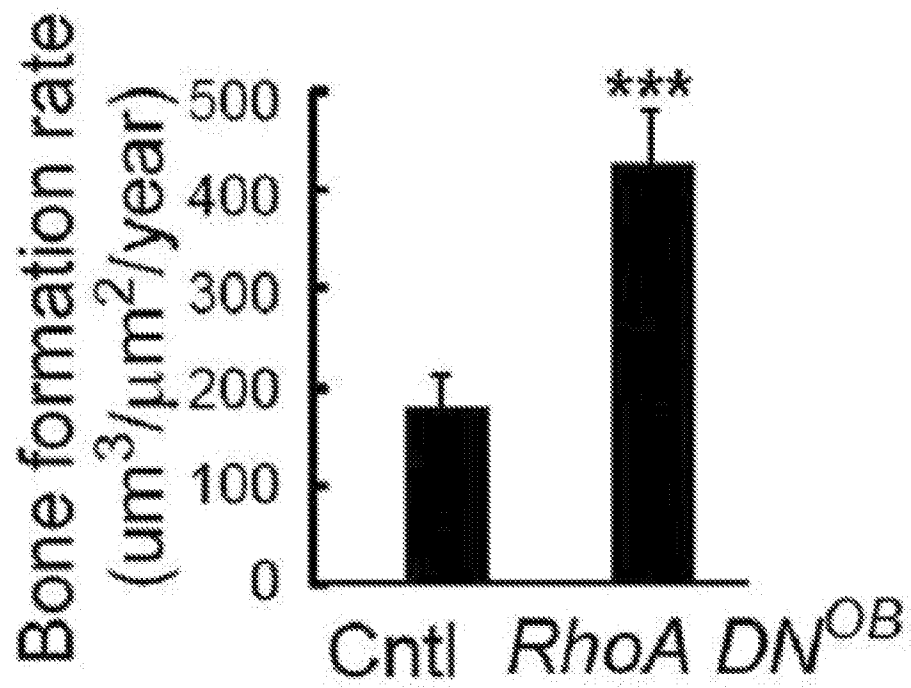


FIGURE 19

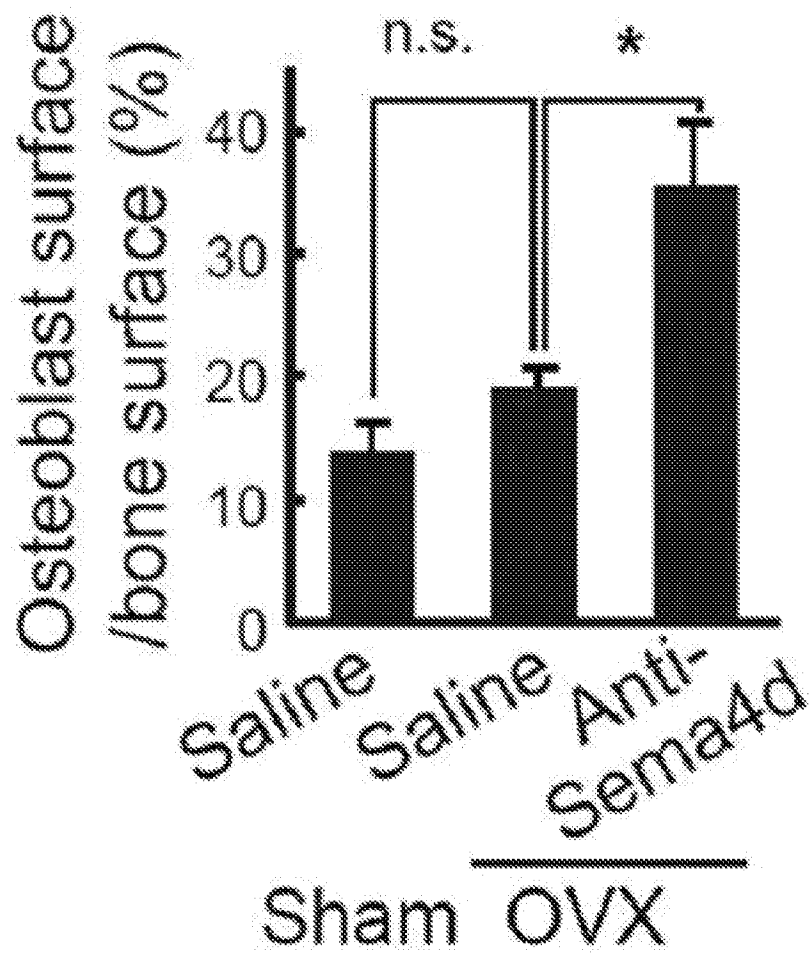


FIGURE 20

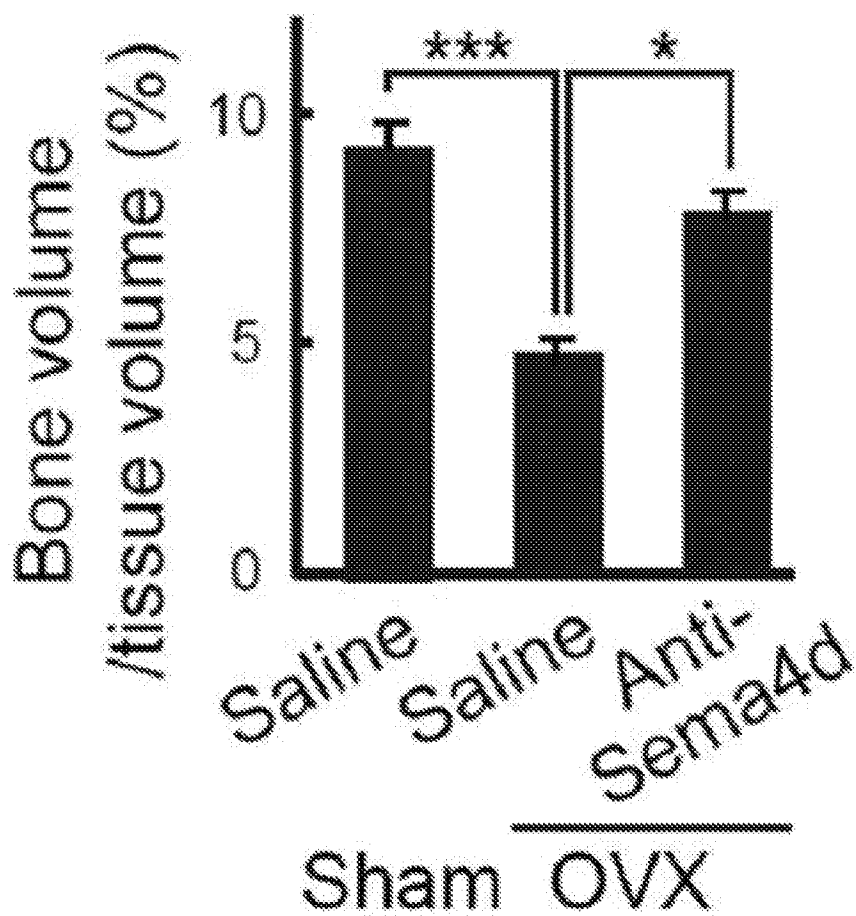


FIGURE 21

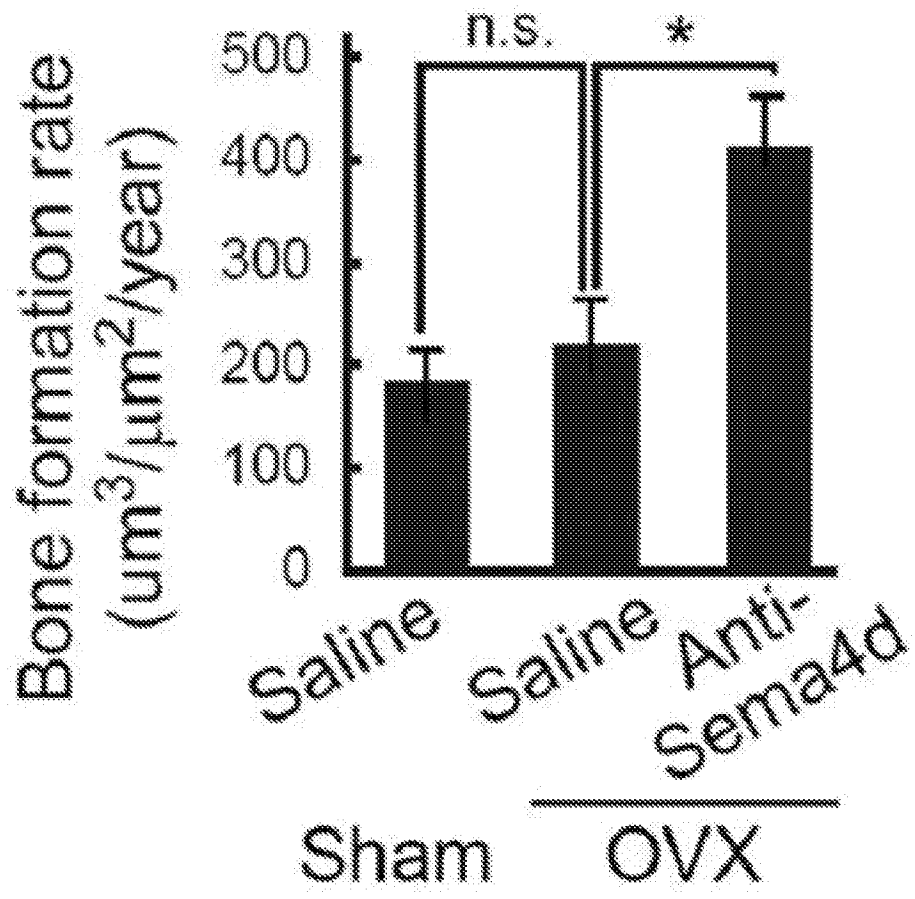


FIGURE 22

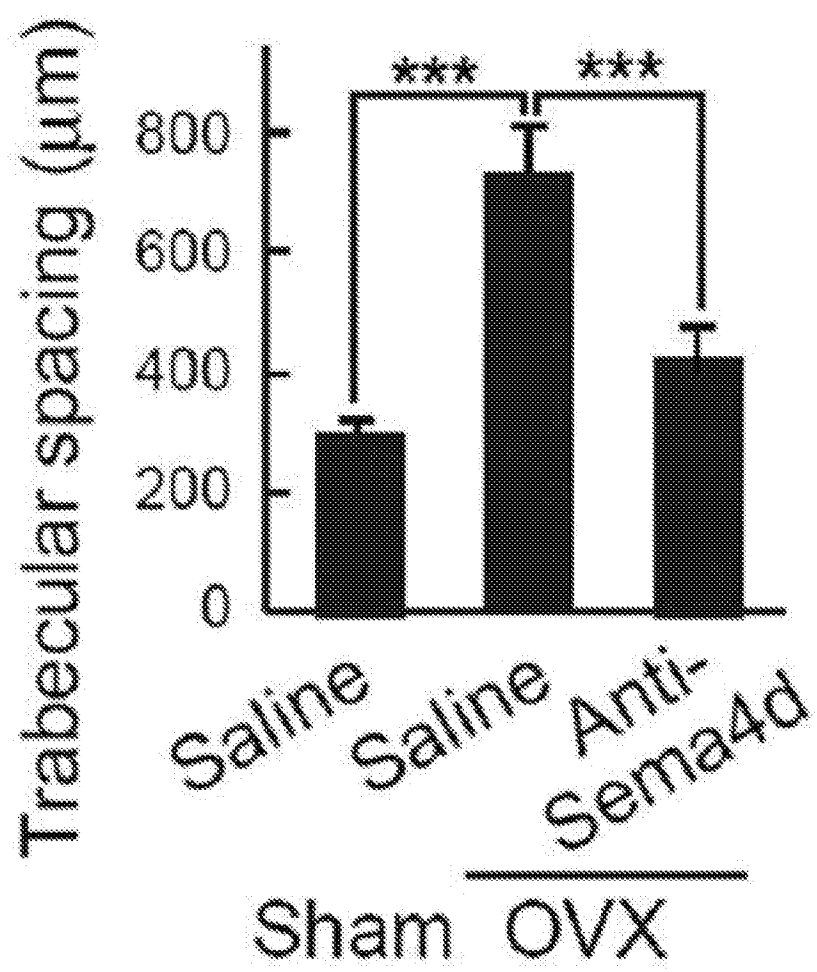
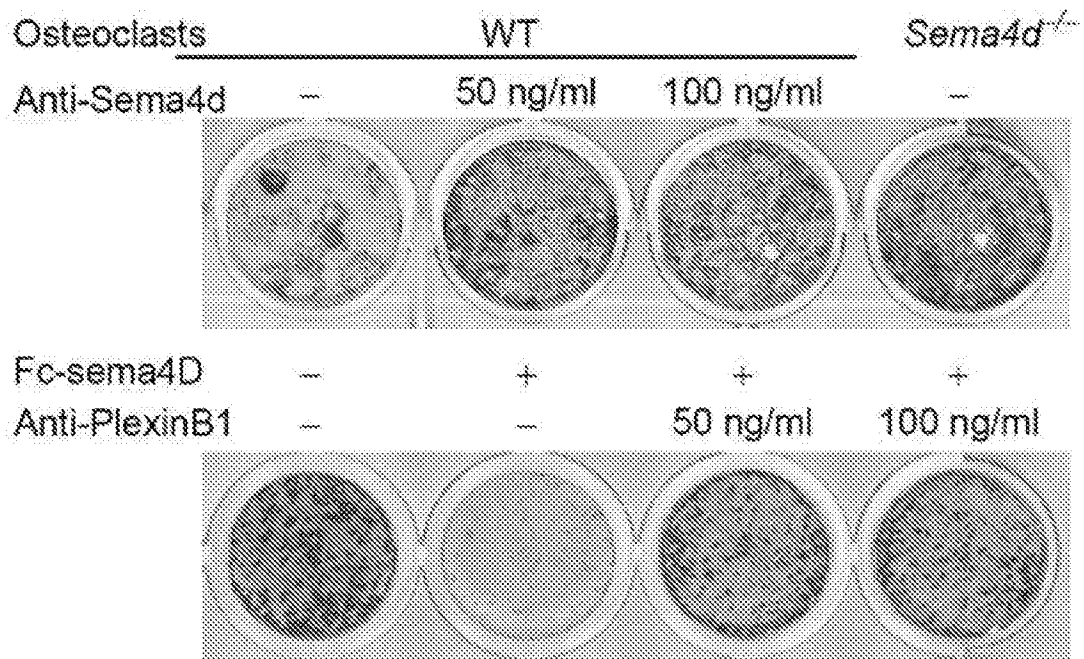


FIGURE 23

**FIGURE 24**

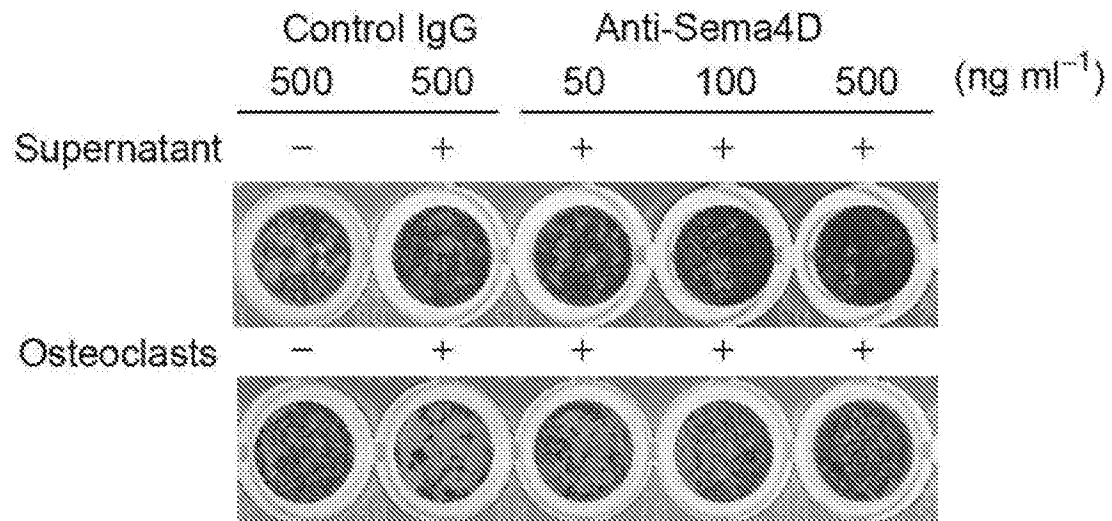


FIGURE 25

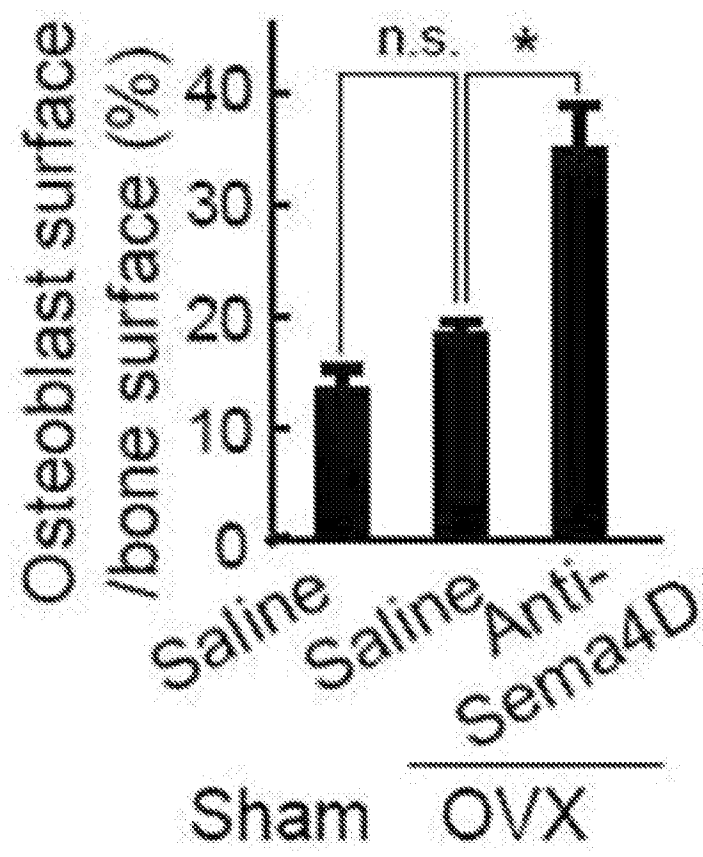


FIGURE 26

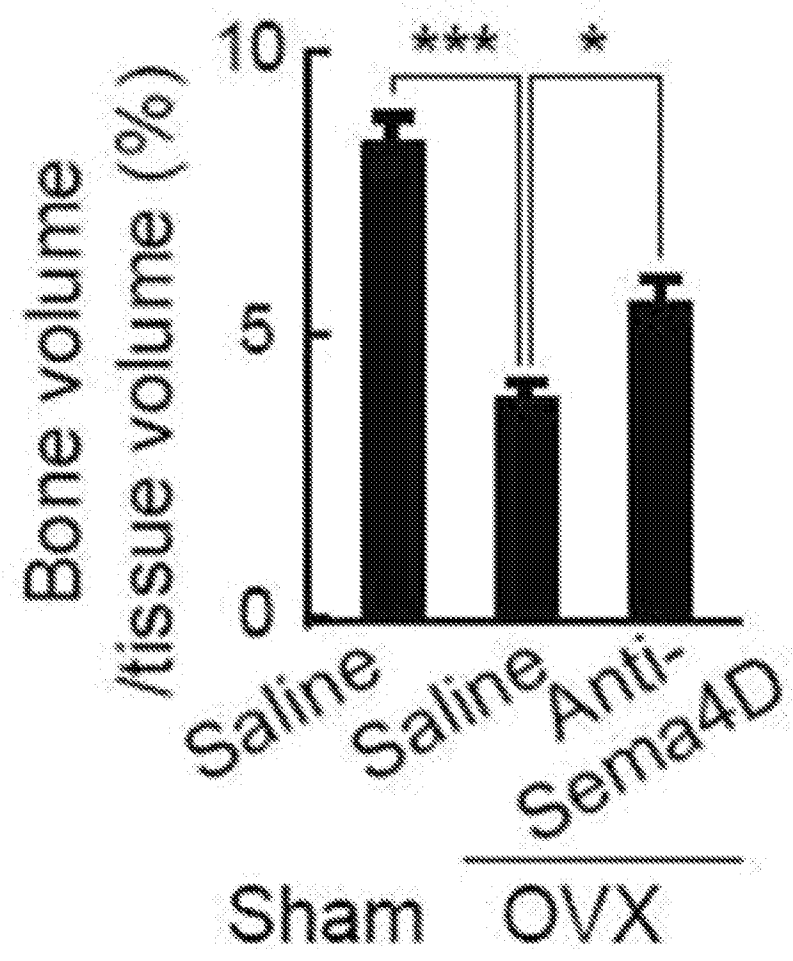


FIGURE 27

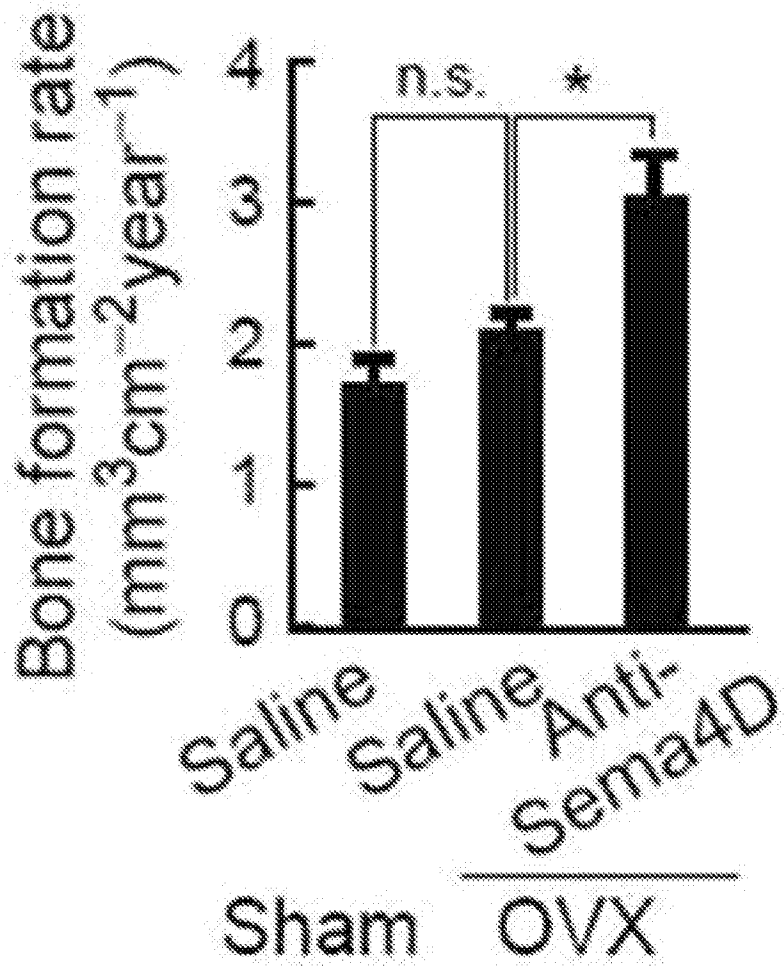


FIGURE 28

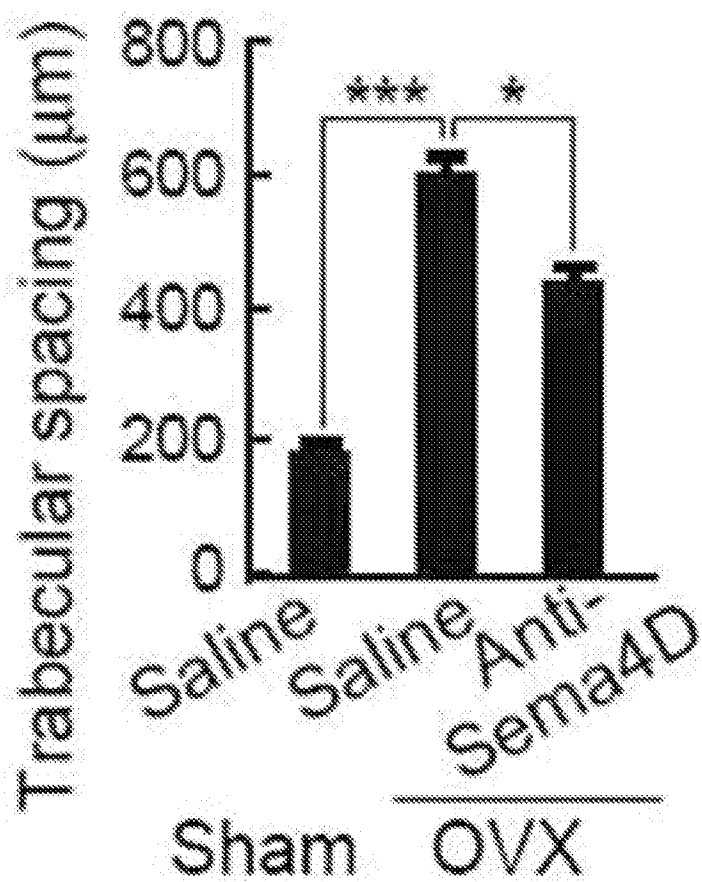


FIGURE 29

JPOXMLDOC01-seql.txt
SEQUENCE LISTING

<110> National University Corporation Tokyo Medical and Dental University
<120> Agent for promoting osteogenesis
<130> P11-005P
<150> 2011-108642
<151> 2011-05-13
<160> 20
<170> PatentIn version 3.1
<210> 1
<211> 2217
<212> DNA
<213> Homo sapiens
<220>
<223> Inventor: Takayanagi, Hiroshi ;Negishi, Takako

```

<400> 1
atgaggatgt gcaccccat tagggggctg ctcatggccc ttgcagtgat gtttgggaca      60
gcgatggcat ttgcacccat accccggatc acctgggagc acagagaggt gcacctggtg      120
cagtttcatg agccagacat ctacaactac tcagccttgc tgctgagcga ggacaaggac      180
accttgatga taggtgcccg ggaggcggtc ttcgctgtga acgcactcaa catctccgag      240
aagcagcatg aggtgtattg gaaggtctca gaagacaaaa aagcaaaatg tgcagaaaag      300
gggaaatcaa aacagacaga gtgcctcaac tacatccggg tgctgcagcc actcagcgcc      360
acttcccttt acgtgtgtgg gaccaacgca ttccagccgg cctgtgacca cctgaactta      420
acatccttta agtttctggg gaaaaatgaa gatggcaaag gaagatgtcc ctttgaccca      480
gcacacagct acacatccgt catggttgat ggagaacttt attcggggac gtcgtataat      540
tttttgggaa gtgaacccat catctcccga aattcttccc acagtcctct gaggacagaa      600
tatgcaatcc cttggctgaa cgagcctagt ttcgtgtttg ctgacgtgat ccgaaaaagc      660
ccagacagcc ccgacggcga ggatgacagg gtctacttct tcttcacgga ggtgtctgtg      720
gagtatgagt ttgtgttcag ggtgctgata ccacggatag caagagtgtg caagggggac      780
cagggcggcc tgaggacctt gcagaagaaa tggacctcct tcctgaaagc ccgactcatc      840
tgctcccggc cagacagcgg cttgggtctt aatgtgctgc gggatgtctt cgtgctcagg      900
tccccgggcc tgaaggtgcc tgtgttctat gactcttca cccacagct gaacaacgtg      960
gggctgtcgg cagtgtgcgc ctacaacctg tccacagccg aggaggtctt ctcccacggg     1020
aagtacatgc agagcaccac agtggagcag tcccacacca agtgggtgcg ctataatggc     1080
ccggtaccca agccgcggcc tggagcgtgc atcgacagcg aggcacgggc cgccaactac     1140
accagtcctt tgaatttgcc agacaagacg ctgcagttcg ttaaagacca ccctttgatg     1200
gatgactcgg taaccccaat agacaacagg cccagggtta tcaagaaaga tgtgaactac     1260
accagatcgg tgggtggaccg gaccagggcc ctggatggga ctgtctatga tgtcatgttt     1320

```

JPOXMLDOC01-seq1.txt

gtcagcacag accgggggagc tctgcacaaa gccatcagcc tcgagcacgc tgttcacatc 1380
atcgaggaga cccagctctt ccaggacttt gagccagtcc agaccctgct gctgtcttca 1440
aagaagggca acaggtttgt ctatgctggc tctaactcgg gcgtgggtcca ggccccgctg 1500
gccttctgtg ggaagcacgg cacctgcgag gactgtgtgc tggcgcgggga cccctactgc 1560
gcctggagcc cgccacagc gacctgcgtg gctctgcacc agaccgagag cccagcagg 1620
ggtttgattc aggagatgag cggcgatgct tctgtgtgcc cggcctcgtc tcctaagccc 1680
ctccctcctc ctggctcctc ttccctgtcc tgtctggggc atgtggggga caggaggctt 1740
tcctctccct ggacccccctg gccagcctcg ggtgcggggc ccgacagcag ctcgagggtc 1800
tccttgctgc cgcccttctt gactgaccag gcacagcacg tgcacgccct ggggaacttc 1860
tacctcttct gccaggccac aggtcctgca gacattcgct ttgtctggga gaagaatggg 1920
cgagctctgg agacctgtgt ccctgtgcag acccatgcac tgcccgatgg caggggccat 1980
gcactcagct ggctgcagga cgccatcagg gaaagcgctg agtatcgctg ctctgtcctc 2040
tcctcagcag ggaacaagac ttcgaaggtg caggttgctg tgatgagacc tgaagtgacc 2100
caccaggaga ggtggaccag agagctctct gcctggaggg ctgtggctgg ggagcacgac 2160
cggatgatgc agagctggag gaaggcgtgg gaaagctgta gcaaggacac cctgtag 2217

<210> 2
<211> 738
<212> PRT
<213> Homo sapiens

<400> 2

Met Arg Met Cys Thr Pro Ile Arg Gly Leu Leu Met Ala Leu Ala Val
1 5 10 15

Met Phe Gly Thr Ala Met Ala Phe Ala Pro Ile Pro Arg Ile Thr Trp
20 25 30

Glu His Arg Glu Val His Leu Val Gln Phe His Glu Pro Asp Ile Tyr
35 40 45

Asn Tyr Ser Ala Leu Leu Leu Ser Glu Asp Lys Asp Thr Leu Tyr Ile
50 55 60

Gly Ala Arg Glu Ala Val Phe Ala Val Asn Ala Leu Asn Ile Ser Glu
65 70 75 80

Lys Gln His Glu Val Tyr Trp Lys Val Ser Glu Asp Lys Lys Ala Lys
85 90 95

Cys Ala Glu Lys Gly Lys Ser Lys Gln Thr Glu Cys Leu Asn Tyr Ile
100 105 110

Arg Val Leu Gln Pro Leu Ser Ala Thr Ser Leu Tyr Val Cys Gly Thr
115 120 125

JPOXMLDOC01-seql.txt

Asn Ala Phe Gln Pro Ala Cys Asp His Leu Asn Leu Thr Ser Phe Lys
 130 135 140
 Phe Leu Gly Lys Asn Glu Asp Gly Lys Gly Arg Cys Pro Phe Asp Pro
 145 150 155 160
 Ala His Ser Tyr Thr Ser Val Met Val Asp Gly Glu Leu Tyr Ser Gly
 165 170 175
 Thr Ser Tyr Asn Phe Leu Gly Ser Glu Pro Ile Ile Ser Arg Asn Ser
 180 185 190
 Ser His Ser Pro Leu Arg Thr Glu Tyr Ala Ile Pro Trp Leu Asn Glu
 195 200 205
 Pro Ser Phe Val Phe Ala Asp Val Ile Arg Lys Ser Pro Asp Ser Pro
 210 215 220
 Asp Gly Glu Asp Asp Arg Val Tyr Phe Phe Phe Thr Glu Val Ser Val
 225 230 235 240
 Glu Tyr Glu Phe Val Phe Arg Val Leu Ile Pro Arg Ile Ala Arg Val
 245 250 255
 Cys Lys Gly Asp Gln Gly Gly Leu Arg Thr Leu Gln Lys Lys Trp Thr
 260 265 270
 Ser Phe Leu Lys Ala Arg Leu Ile Cys Ser Arg Pro Asp Ser Gly Leu
 275 280 285
 Val Phe Asn Val Leu Arg Asp Val Phe Val Leu Arg Ser Pro Gly Leu
 290 295 300
 Lys Val Pro Val Phe Tyr Ala Leu Phe Thr Pro Gln Leu Asn Asn Val
 305 310 315 320
 Gly Leu Ser Ala Val Cys Ala Tyr Asn Leu Ser Thr Ala Glu Glu Val
 325 330 335
 Phe Ser His Gly Lys Tyr Met Gln Ser Thr Thr Val Glu Gln Ser His
 340 345 350
 Thr Lys Trp Val Arg Tyr Asn Gly Pro Val Pro Lys Pro Arg Pro Gly
 355 360 365
 Ala Cys Ile Asp Ser Glu Ala Arg Ala Ala Asn Tyr Thr Ser Ser Leu
 370 375 380
 Asn Leu Pro Asp Lys Thr Leu Gln Phe Val Lys Asp His Pro Leu Met
 385 390 395 400

JPOXMLDOC01-seql.txt

Asp Asp Ser Val Thr Pro Ile Asp Asn Arg Pro Arg Leu Ile Lys Lys
 405 410 415
 Asp Val Asn Tyr Thr Gln Ile Val Val Asp Arg Thr Gln Ala Leu Asp
 420 425 430
 Gly Thr Val Tyr Asp Val Met Phe Val Ser Thr Asp Arg Gly Ala Leu
 435 440 445
 His Lys Ala Ile Ser Leu Glu His Ala Val His Ile Ile Glu Glu Thr
 450 455 460
 Gln Leu Phe Gln Asp Phe Glu Pro Val Gln Thr Leu Leu Leu Ser Ser
 465 470 475 480
 Lys Lys Gly Asn Arg Phe Val Tyr Ala Gly Ser Asn Ser Gly Val Val
 485 490 495
 Gln Ala Pro Leu Ala Phe Cys Gly Lys His Gly Thr Cys Glu Asp Cys
 500 505 510
 Val Leu Ala Arg Asp Pro Tyr Cys Ala Trp Ser Pro Pro Thr Ala Thr
 515 520 525
 Cys Val Ala Leu His Gln Thr Glu Ser Pro Ser Arg Gly Leu Ile Gln
 530 535 540
 Glu Met Ser Gly Asp Ala Ser Val Cys Pro Ala Ser Ser Pro Lys Pro
 545 550 555 560
 Leu Pro Pro Pro Gly Ser Ser Ser Leu Ser Cys Leu Gly His Val Gly
 565 570 575
 Asp Arg Arg Leu Ser Ser Pro Trp Thr Pro Trp Pro Ala Ser Gly Ala
 580 585 590
 Gly Pro Asp Ser Ser Ser Arg Val Ser Leu Leu Pro Pro Phe Leu Ser
 595 600 605
 Asp Gln Ala Gln His Val His Ala Leu Gly Asn Phe Tyr Leu Phe Cys
 610 615 620
 Gln Ala Thr Gly Pro Ala Asp Ile Arg Phe Val Trp Glu Lys Asn Gly
 625 630 635 640
 Arg Ala Leu Glu Thr Cys Val Pro Val Gln Thr His Ala Leu Pro Asp
 645 650 655
 Gly Arg Ala His Ala Leu Ser Trp Leu Gln Asp Ala Ile Arg Glu Ser
 660 665 670

JPOXMLDOC01-seql.txt

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

Lys Val Gln Val Ala Val Met Arg Pro Glu Val Thr His Gln Glu Arg
690 695 700

Trp Thr Arg Glu Leu Ser Ala Trp Arg Ala Val Ala Gly Glu His Asp
705 710 715 720

Arg Met Met Gln Ser Trp Arg Lys Ala Trp Glu Ser Cys Ser Lys Asp
725 730 735

Thr Leu

<210> 3
<211> 6408
<212> DNA
<213> Homo sapiens

<400> 3
atgcctgctc tgggcccagc tcttctccag gctctctggg ccgggtgggt cctcaccctc 60
cagccccttc caccaactgc attcactccc aatggcacgt atctgcagca cctggcaagg 120
gacccacact caggcacct ctacctgggg gctaccaact tcctgttcca gctgagccct 180
gggctgcagc tggaggccac agtgtccacc ggccctgtgc tagacagcag ggactgcctg 240
ccacctgtga tgcctgatga gtgccccag gccagccta ccaacaacc gaatcagctg 300
ctcctggtga gccaggggc cctggtggtg tgcgggagcg tgcaccaggg ggtctgtgaa 360
cagcggcgcc tggggcagct cgagcagctg ctgctgcggc cagagcggcc tggggacaca 420
caatatgtgg ctgccaatga tcctgcggtc agcacggtgg ggctggtagc ccagggcttg 480
gcaggggagc ccctcctgtt tgtggggcga ggatacacca gcaggggtgt ggggggtggc 540
attccacca tcacaaccg ggccctgtgg ccgcccagacc cccaagctgc cttctcctat 600
gaggagacag ccaagctggc agtgggccgc ctctccgagt acagccacca cttcgtgagt 660
gcctttgcac gtggggccag cgcctacttc ctgttcctgc ggcgggacct gcaggctcag 720
tctagagctt ttcgtgccta tgtatctcga gtgtgtctcc gggaccagca ctactactcc 780
tatgtggagt tgcctctggc ctgcgaagg ggccgctacg ggctgatcca ggctgcagct 840
gtggccacgt ccagggaggt ggcgcatggg gaggtgctct ttgcagcttt ctctcggct 900
gcacccccca ctgtgggccg gccccatcg gcggctgctg gggcatctgg agcctctgcc 960
ctctgtgcct tccccctgga tgagggtgac cggcttgcta atcgcacgcg agatgcctgc 1020
tacaccggg agggctgtgc tgaggatggg accgaggtgg cctacatcga gtatgatgtc 1080
aattctgact gtgcacagct gccagtggac accctggatg cttatccctg tggctcagac 1140
cacacgcca gccccatggc cagccgggtc ccgctggaag ccacaccaat tctggagtgg 1200

JPOXMLDOC01-seq1.txt

ccagggattc	agctaacagc	tgtggcagtc	accatggaag	atggacacac	catcgctttc	1260
ctgggtgata	gtcaaggga	gctgcacagg	gtctacttgg	gccagggag	cgatggccac	1320
ccatactcca	cacagagcat	ccagcagggg	tctgcagtga	gcagagacct	cacctttgat	1380
gggacctttg	agcacctgta	tgtcatgacc	cagagcacac	ttctgaaggt	tcctgtggct	1440
tcctgtgctc	agcacctgga	ctgtgcatct	tgcttgctc	acagggaccc	atactgtggg	1500
tggtgctg	tccttggcag	gtgcagtcgc	cgttctgagt	gctcgagggg	ccagggccca	1560
gagcagtggc	tatggagctt	ccagcctgag	ctgggctgtc	tgcaagtggc	agccatgagt	1620
cctgccaaaca	tcagccgaga	ggagacgagg	gaggttttcc	tatcagtgcc	agacctgccca	1680
cccctgtggc	caggggagtc	atattcctgc	cactttgggg	aacatcagag	tcctgccctg	1740
ctgactgggt	ctgggtgtgat	gtgcccctcc	ccagacccta	gtgaggcccc	agtgtgtccg	1800
agaggagccg	actacgtatc	cgtgagcgtg	gagctcagat	ttggcgctgt	tgtgatcgcc	1860
aaaacttccc	tctctttcta	tgactgtgtg	gcggtcactg	aactccgccc	atctgcgcag	1920
tgccaggcct	gtgtgagcag	ccgctggggg	tgtaactggt	gtgtctggca	gcacctgtgc	1980
accacaaagg	cctcgtgtga	tgctgggccc	atggttgcaa	gccatcagag	cccgtttgtc	2040
tccccagacc	ctcctgcaag	aggtggaccc	agcccctccc	caccacagc	ccccaaagcc	2100
ctggccaccc	ctgctcctga	cacccttccc	gtggagcctg	gggctccctc	cacagccaca	2160
gcttcggaca	tctcacctgg	ggctagtcct	tcctgtctca	gcccctgggg	gccatgggca	2220
ggttctggct	ccatatcttc	ccctggctcc	acagggtcgc	ctctccatga	ggagccctcc	2280
cctcccagcc	ccaaaatgg	acctggaacc	gctgtccctg	ccccactga	cttcagaccc	2340
tcagccacac	ctgaggacct	cttggcctcc	ccgctgtcac	cgtcagaggt	agcagcagtg	2400
ccccctgcag	accctggccc	cgaggctctt	catcccacag	tgcccctgga	cctgccccct	2460
gccactgttc	ctgccaccac	tttcccaggg	gccatgggct	ccgtgaagcc	cgccctggac	2520
tggctcacga	gagaaggcgg	cgagctgccc	gaggcggacg	agtggacggg	gggtgacgca	2580
ccgccttct	ccacttccac	cctcctctca	ggtgatggag	actcagcaga	gcttgagggc	2640
cctcccgccc	ccctatcct	cccgtccagc	ctcgactacc	agtatgacac	ccccgggctc	2700
tgggagctgg	aagaggcgac	cttgggggca	agctcctgcc	cctgtgtgga	gagcgttcag	2760
ggctccacgt	tgatgccggt	ccatgtggag	cgggaaatcc	ggctgctagg	caggaacctg	2820
caccttttcc	aggatggccc	aggagacaat	gagtgtgtga	tggagctgga	gggcctcgag	2880
gtggtgggtg	aggcccggt	cgagtgtgag	ccacctccag	ataccagtg	ccatgtcacc	2940
tgccagcagc	accagctcag	ctatgaggct	ctgcagccgg	agctccgtgt	ggggctgttt	3000
ctgcgtcggg	ccggccgtct	gcgtgtggac	agtgtgagg	ggctgcatgt	ggtactgtat	3060
gactgttccg	tgggacatgg	agactgcagc	cgctgccaaa	ctgccatgcc	ccagtatggc	3120
tgtgtgtggt	gtgaggggga	gcgtccacgt	tgtgtgaccc	gggaggcctg	tggtgaggct	3180
gaggctgtgg	ccaccagtg	cccagcgccc	ctcatccact	cgggtggagcc	actgactggg	3240

JPOXMLDOC01-seq1.txt

cctgtagacg	gaggcaccgc	tgtcaccatc	aggggctcca	acctgggcca	gcatgtgcag	3300
gatgtgctgg	gcatggtcac	ggtggctgga	gtgccctgtg	ctgtggatgc	ccaggagtac	3360
gaggtctcca	gcagcctcgt	gtgcatcacc	ggggccagtg	gggaggaggt	ggccggcgcc	3420
acagcgggtg	aggtgccggg	aagaggacgt	ggtgtctcag	aacacgactt	tgcctaccag	3480
gatccgaagg	tccattccat	cttcccggcc	cgcgggccca	gagctggggg	caccctcttc	3540
accctgaatg	gctccaagct	cctgactggg	cggctggagg	acatccgagt	ggtgggttga	3600
gaccagcctt	gtcacttgct	gccggagcag	cagtcagaac	aactgcggtg	tgagaccagc	3660
ccacgcccc	cgctgccac	gctccctgtg	gctgtgtggt	ttggggccac	ggagcggagg	3720
cttcaacgcg	gacagttcaa	gtataccttg	gacccaaca	tcacctctgc	tggccccacc	3780
aagagcttcc	tcagtggagg	acgtgagata	tgcgtccgtg	gccagaatct	ggacgtggta	3840
cagacgcaa	gaatccgggt	gaccgtggtc	tcgagaatgc	tgacgcccag	ccaggggctt	3900
ggacggaggc	gtcgcgtggt	cccggagacg	gcatgttccc	ttggaccctc	ctgcagtagc	3960
cagcaatttg	aggagccgtg	ccatgtcaac	tcctcccagc	tcatcacgtg	ccgcacacct	4020
gccctcccag	gcctgcctga	ggacccctgg	gtccgggtgg	aatttatact	tgacaacctg	4080
gtctttgact	ttgcaacct	gaacccaca	cctttctcct	atgaggccga	ccccaccctg	4140
cagccactca	accctgagga	ccccaccatg	ccattccggc	acaagcctgg	gagtgtgttc	4200
tccgtggagg	gggagaacct	ggaccttgca	atgtccaagg	aggaggtggt	ggctatgata	4260
ggggatggcc	cctgtgtggt	gaagacgctg	acgcggcacc	acctgtactg	cgagcccccc	4320
gtggagcagc	ccctgccacg	gcaccatgcc	ctccgagagg	cacctgactc	tttgccctgag	4380
ttcacggtgc	agatggggaa	cttgcgcttc	tccttgggtc	acgtgcagta	tgacggcgag	4440
agccctgggg	cttttctgt	ggcagcccag	gtgggcttgg	gggtgggcac	ctctcttctg	4500
gctctgggtg	tcacatcat	tgtcctcatg	tacaggagga	agagcaagca	ggccctgagg	4560
gactataaga	aggttcagat	ccagctggag	aatctggaga	gcagtgtgcg	ggaccgctgc	4620
aagaaggaat	tcacagacct	catgactgag	atgaccgatc	tcaccagtga	cctcctgggc	4680
agcggcatcc	ccttcctcga	ctacaagggt	tatgcggaga	ggatcttctt	ccctgggcac	4740
cgcgagtcgc	ccttgaccgc	ggacctgggt	gtgcctgaga	gcagacggcc	cactgtggag	4800
caagggctgg	ggcagctctc	taacctgctc	aacagcaagc	tcttcctcac	caagttcatc	4860
cacacgctgg	agagccagcg	caccttttca	gctcgggacc	gtgcctacgt	ggcatctctg	4920
ctcaccgtgg	cactgcatgg	gaagcttgag	tatttctactg	acatcctccg	cactctgctc	4980
agtgacctgg	ttgcccagta	tgtggccaag	aacccaagc	tgatgctgcg	caggacagag	5040
actgtggtgg	agaagctgct	caccaactgg	atgtccatct	gtctgtatac	cttcgtgagg	5100
gactccgtag	gggagcctct	gtacatgctc	tttcgagggg	ttaagcacca	agtgataag	5160
gggccagtgg	acagtgtgac	aggcaaggcc	aaatacacct	tgaacgacaa	ccgcctgctc	5220
agagaggatg	tggagtaccg	tcccctgacc	ttgaatgcac	tattggctgt	ggggcctggg	5280

JPOXMLDOC01-seql.txt

gcaggagagg cccagggcgt gcccggtgaag gtcctagact gtgacaccat ctcccaggca 5340
aaggagaaga tgctggacca gctttataaa ggagtgcctc tcacccagcg gccagaccct 5400
cgcacccttg atgttgagtg gcggtctggg gtggccgggc acctcattct ttctgacgag 5460
gatgtcactt ctgaggtcca gggctctgtg aggcgcctga acacactgca gcattacaag 5520
gtcccagatg gagcaactgt ggccctcgtc ccctgcctca ccaagcatgt gtcccgggaa 5580
aaccaggatt atgtccctgg agagcggacc ccaatgctgg aggatgtaga tgaggggggc 5640
atccggccct ggcacctggt gaagccaagt gatgagccgg agccgcccag gcctcggagg 5700
ggcagccttc ggggcgggga gcgtgagcgc gccaaaggcca tccctgagat ctacctgacc 5760
cgcctgctgt ccatgaaggg caccctgcag aagttcgtgg atgacctgtt ccagggtgatt 5820
ctcagcacca gccgccccgt gccgctcgt gtgaagtact tctttgacct gctggatgag 5880
caggcccagc agcatggcat ctccgaccag gacaccatcc acatctggaa gaccaacagc 5940
ttgcctctga ggttctggat caatataata aaaaacccgc agtttgtgtt cgacgtgcaa 6000
acatctgata acatggatgc ggtgctcctt gtcattgcac agaccttcat ggacgcctgc 6060
accctggccg accacaagct gggccgggac tccccgatca acaaacttct gtatgcacgg 6120
gacattcccc ggtacaagcg gatggtggaa aggtactatg cagacatcag acagactgtc 6180
ccagccagcg accaagagat gaactctgtc ctggctgaac tgtcctggaa ctactccgga 6240
gacctcgggg cgcgagtggc cctgcatgaa ctctacaagt acatcaacaa gtactatgac 6300
cagatcatca ctgccctgga ggaggatggc acggcccaga agatgcagct gggctatcgg 6360
ctccagcaga ttgcagctgc tgtggaaaac aaggtcacag atctatag 6408

<210> 4
<211> 2135
<212> PRT
<213> Homo sapiens

<400> 4

Met Pro Ala Leu Gly Pro Ala Leu Leu Gln Ala Leu Trp Ala Gly Trp
1 5 10 15

Val Leu Thr Leu Gln Pro Leu Pro Pro Thr Ala Phe Thr Pro Asn Gly
20 25 30

Thr Tyr Leu Gln His Leu Ala Arg Asp Pro Thr Ser Gly Thr Leu Tyr
35 40 45

Leu Gly Ala Thr Asn Phe Leu Phe Gln Leu Ser Pro Gly Leu Gln Leu
50 55 60

Glu Ala Thr Val Ser Thr Gly Pro Val Leu Asp Ser Arg Asp Cys Leu
65 70 75 80

Pro Pro Val Met Pro Asp Glu Cys Pro Gln Ala Gln Pro Thr Asn Asn
85 90 95

JPOXMLDOC01-seql.txt

Pro Asn Gln Leu Leu Leu Val Ser Pro Gly Ala Leu Val Val Cys Gly
100 105 110

Ser Val His Gln Gly Val Cys Glu Gln Arg Arg Leu Gly Gln Leu Glu
115 120 125

Gln Leu Leu Leu Arg Pro Glu Arg Pro Gly Asp Thr Gln Tyr Val Ala
130 135 140

Ala Asn Asp Pro Ala Val Ser Thr Val Gly Leu Val Ala Gln Gly Leu
145 150 155 160

Ala Gly Glu Pro Leu Leu Phe Val Gly Arg Gly Tyr Thr Ser Arg Gly
165 170 175

Val Gly Gly Gly Ile Pro Pro Ile Thr Thr Arg Ala Leu Trp Pro Pro
180 185 190

Asp Pro Gln Ala Ala Phe Ser Tyr Glu Glu Thr Ala Lys Leu Ala Val
195 200 205

Gly Arg Leu Ser Glu Tyr Ser His His Phe Val Ser Ala Phe Ala Arg
210 215 220

Gly Ala Ser Ala Tyr Phe Leu Phe Leu Arg Arg Asp Leu Gln Ala Gln
225 230 235 240

Ser Arg Ala Phe Arg Ala Tyr Val Ser Arg Val Cys Leu Arg Asp Gln
245 250 255

His Tyr Tyr Ser Tyr Val Glu Leu Pro Leu Ala Cys Glu Gly Gly Arg
260 265 270

Tyr Gly Leu Ile Gln Ala Ala Ala Val Ala Thr Ser Arg Glu Val Ala
275 280 285

His Gly Glu Val Leu Phe Ala Ala Phe Ser Ser Ala Ala Pro Pro Thr
290 295 300

Val Gly Arg Pro Pro Ser Ala Ala Ala Gly Ala Ser Gly Ala Ser Ala
305 310 315 320

Leu Cys Ala Phe Pro Leu Asp Glu Val Asp Arg Leu Ala Asn Arg Thr
325 330 335

Arg Asp Ala Cys Tyr Thr Arg Glu Gly Arg Ala Glu Asp Gly Thr Glu
340 345 350

Val Ala Tyr Ile Glu Tyr Asp Val Asn Ser Asp Cys Ala Gln Leu Pro
355 360 365

JPOXMLDOC01-seql.txt

Val Asp Thr Leu Asp Ala Tyr Pro Cys Gly Ser Asp His Thr Pro Ser
370 375 380

Pro Met Ala Ser Arg Val Pro Leu Glu Ala Thr Pro Ile Leu Glu Trp
385 390 395 400

Pro Gly Ile Gln Leu Thr Ala Val Ala Val Thr Met Glu Asp Gly His
405 410 415

Thr Ile Ala Phe Leu Gly Asp Ser Gln Gly Gln Leu His Arg Val Tyr
420 425 430

Leu Gly Pro Gly Ser Asp Gly His Pro Tyr Ser Thr Gln Ser Ile Gln
435 440 445

Gln Gly Ser Ala Val Ser Arg Asp Leu Thr Phe Asp Gly Thr Phe Glu
450 455 460

His Leu Tyr Val Met Thr Gln Ser Thr Leu Leu Lys Val Pro Val Ala
465 470 475 480

Ser Cys Ala Gln His Leu Asp Cys Ala Ser Cys Leu Ala His Arg Asp
485 490 495

Pro Tyr Cys Gly Trp Cys Val Leu Leu Gly Arg Cys Ser Arg Arg Ser
500 505 510

Glu Cys Ser Arg Gly Gln Gly Pro Glu Gln Trp Leu Trp Ser Phe Gln
515 520 525

Pro Glu Leu Gly Cys Leu Gln Val Ala Ala Met Ser Pro Ala Asn Ile
530 535 540

Ser Arg Glu Glu Thr Arg Glu Val Phe Leu Ser Val Pro Asp Leu Pro
545 550 555 560

Pro Leu Trp Pro Gly Glu Ser Tyr Ser Cys His Phe Gly Glu His Gln
565 570 575

Ser Pro Ala Leu Leu Thr Gly Ser Gly Val Met Cys Pro Ser Pro Asp
580 585 590

Pro Ser Glu Ala Pro Val Leu Pro Arg Gly Ala Asp Tyr Val Ser Val
595 600 605

Ser Val Glu Leu Arg Phe Gly Ala Val Val Ile Ala Lys Thr Ser Leu
610 615 620

Ser Phe Tyr Asp Cys Val Ala Val Thr Glu Leu Arg Pro Ser Ala Gln
625 630 635 640

JPOXMLDOC01-seql.txt

Cys Gln Ala Cys Val Ser Ser Arg Trp Gly Cys Asn Trp Cys Val Trp
 645 650 655
 Gln His Leu Cys Thr His Lys Ala Ser Cys Asp Ala Gly Pro Met Val
 660 665 670
 Ala Ser His Gln Ser Pro Leu Val Ser Pro Asp Pro Pro Ala Arg Gly
 675 680 685
 Gly Pro Ser Pro Ser Pro Pro Thr Ala Pro Lys Ala Leu Ala Thr Pro
 690 695 700
 Ala Pro Asp Thr Leu Pro Val Glu Pro Gly Ala Pro Ser Thr Ala Thr
 705 710 715 720
 Ala Ser Asp Ile Ser Pro Gly Ala Ser Pro Ser Leu Leu Ser Pro Trp
 725 730 735
 Gly Pro Trp Ala Gly Ser Gly Ser Ile Ser Ser Pro Gly Ser Thr Gly
 740 745 750
 Ser Pro Leu His Glu Glu Pro Ser Pro Pro Ser Pro Gln Asn Gly Pro
 755 760 765
 Gly Thr Ala Val Pro Ala Pro Thr Asp Phe Arg Pro Ser Ala Thr Pro
 770 775 780
 Glu Asp Leu Leu Ala Ser Pro Leu Ser Pro Ser Glu Val Ala Ala Val
 785 790 795 800
 Pro Pro Ala Asp Pro Gly Pro Glu Ala Leu His Pro Thr Val Pro Leu
 805 810 815
 Asp Leu Pro Pro Ala Thr Val Pro Ala Thr Thr Phe Pro Gly Ala Met
 820 825 830
 Gly Ser Val Lys Pro Ala Leu Asp Trp Leu Thr Arg Glu Gly Gly Glu
 835 840 845
 Leu Pro Glu Ala Asp Glu Trp Thr Gly Gly Asp Ala Pro Ala Phe Ser
 850 855 860
 Thr Ser Thr Leu Leu Ser Gly Asp Gly Asp Ser Ala Glu Leu Glu Gly
 865 870 875 880
 Pro Pro Ala Pro Leu Ile Leu Pro Ser Ser Leu Asp Tyr Gln Tyr Asp
 885 890 895
 Thr Pro Gly Leu Trp Glu Leu Glu Glu Ala Thr Leu Gly Ala Ser Ser
 900 905 910

JPOXMLDOC01-seql.txt

Cys Pro Cys Val Glu Ser Val Gln Gly Ser Thr Leu Met Pro Val His
 915 920 925
 Val Glu Arg Glu Ile Arg Leu Leu Gly Arg Asn Leu His Leu Phe Gln
 930 935 940
 Asp Gly Pro Gly Asp Asn Glu Cys Val Met Glu Leu Glu Gly Leu Glu
 945 950 955 960
 Val Val Val Glu Ala Arg Val Glu Cys Glu Pro Pro Pro Asp Thr Gln
 965 970 975
 Cys His Val Thr Cys Gln Gln His Gln Leu Ser Tyr Glu Ala Leu Gln
 980 985 990
 Pro Glu Leu Arg Val Gly Leu Phe Leu Arg Arg Ala Gly Arg Leu Arg
 995 1000 1005
 Val Asp Ser Ala Glu Gly Leu His Val Val Leu Tyr Asp Cys Ser
 1010 1015 1020
 Val Gly His Gly Asp Cys Ser Arg Cys Gln Thr Ala Met Pro Gln
 1025 1030 1035
 Tyr Gly Cys Val Trp Cys Glu Gly Glu Arg Pro Arg Cys Val Thr
 1040 1045 1050
 Arg Glu Ala Cys Gly Glu Ala Glu Ala Val Ala Thr Gln Cys Pro
 1055 1060 1065
 Ala Pro Leu Ile His Ser Val Glu Pro Leu Thr Gly Pro Val Asp
 1070 1075 1080
 Gly Gly Thr Arg Val Thr Ile Arg Gly Ser Asn Leu Gly Gln His
 1085 1090 1095
 Val Gln Asp Val Leu Gly Met Val Thr Val Ala Gly Val Pro Cys
 1100 1105 1110
 Ala Val Asp Ala Gln Glu Tyr Glu Val Ser Ser Ser Leu Val Cys
 1115 1120 1125
 Ile Thr Gly Ala Ser Gly Glu Glu Val Ala Gly Ala Thr Ala Val
 1130 1135 1140
 Glu Val Pro Gly Arg Gly Arg Gly Val Ser Glu His Asp Phe Ala
 1145 1150 1155
 Tyr Gln Asp Pro Lys Val His Ser Ile Phe Pro Ala Arg Gly Pro
 1160 1165 1170

JPOXMLDOC01-seql.txt

Arg	Ala	Gly	Gly	Thr	Arg	Leu	Thr	Leu	Asn	Gly	Ser	Lys	Leu	Leu
	1175					1180					1185			
Thr	Gly	Arg	Leu	Glu	Asp	Ile	Arg	Val	Val	Val	Gly	Asp	Gln	Pro
	1190					1195					1200			
Cys	His	Leu	Leu	Pro	Glu	Gln	Gln	Ser	Glu	Gln	Leu	Arg	Cys	Glu
	1205					1210					1215			
Thr	Ser	Pro	Arg	Pro	Thr	Pro	Ala	Thr	Leu	Pro	Val	Ala	Val	Trp
	1220					1225					1230			
Phe	Gly	Ala	Thr	Glu	Arg	Arg	Leu	Gln	Arg	Gly	Gln	Phe	Lys	Tyr
	1235					1240					1245			
Thr	Leu	Asp	Pro	Asn	Ile	Thr	Ser	Ala	Gly	Pro	Thr	Lys	Ser	Phe
	1250					1255					1260			
Leu	Ser	Gly	Gly	Arg	Glu	Ile	Cys	Val	Arg	Gly	Gln	Asn	Leu	Asp
	1265					1270					1275			
Val	Val	Gln	Thr	Pro	Arg	Ile	Arg	Val	Thr	Val	Val	Ser	Arg	Met
	1280					1285					1290			
Leu	Gln	Pro	Ser	Gln	Gly	Leu	Gly	Arg	Arg	Arg	Arg	Val	Val	Pro
	1295					1300					1305			
Glu	Thr	Ala	Cys	Ser	Leu	Gly	Pro	Ser	Cys	Ser	Ser	Gln	Gln	Phe
	1310					1315					1320			
Glu	Glu	Pro	Cys	His	Val	Asn	Ser	Ser	Gln	Leu	Ile	Thr	Cys	Arg
	1325					1330					1335			
Thr	Pro	Ala	Leu	Pro	Gly	Leu	Pro	Glu	Asp	Pro	Trp	Val	Arg	Val
	1340					1345					1350			
Glu	Phe	Ile	Leu	Asp	Asn	Leu	Val	Phe	Asp	Phe	Ala	Thr	Leu	Asn
	1355					1360					1365			
Pro	Thr	Pro	Phe	Ser	Tyr	Glu	Ala	Asp	Pro	Thr	Leu	Gln	Pro	Leu
	1370					1375					1380			
Asn	Pro	Glu	Asp	Pro	Thr	Met	Pro	Phe	Arg	His	Lys	Pro	Gly	Ser
	1385					1390					1395			
Val	Phe	Ser	Val	Glu	Gly	Glu	Asn	Leu	Asp	Leu	Ala	Met	Ser	Lys
	1400					1405					1410			
Glu	Glu	Val	Val	Ala	Met	Ile	Gly	Asp	Gly	Pro	Cys	Val	Val	Lys
	1415					1420					1425			

JPOXMLDOC01-seql.txt

Thr	Leu	Thr	Arg	His	His	Leu	Tyr	Cys	Glu	Pro	Pro	Val	Glu	Gln
	1430					1435					1440			
Pro	Leu	Pro	Arg	His	His	Ala	Leu	Arg	Glu	Ala	Pro	Asp	Ser	Leu
	1445					1450					1455			
Pro	Glu	Phe	Thr	Val	Gln	Met	Gly	Asn	Leu	Arg	Phe	Ser	Leu	Gly
	1460					1465					1470			
His	Val	Gln	Tyr	Asp	Gly	Glu	Ser	Pro	Gly	Ala	Phe	Pro	Val	Ala
	1475					1480					1485			
Ala	Gln	Val	Gly	Leu	Gly	Val	Gly	Thr	Ser	Leu	Leu	Ala	Leu	Gly
	1490					1495					1500			
Val	Ile	Ile	Ile	Val	Leu	Met	Tyr	Arg	Arg	Lys	Ser	Lys	Gln	Ala
	1505					1510					1515			
Leu	Arg	Asp	Tyr	Lys	Lys	Val	Gln	Ile	Gln	Leu	Glu	Asn	Leu	Glu
	1520					1525					1530			
Ser	Ser	Val	Arg	Asp	Arg	Cys	Lys	Lys	Glu	Phe	Thr	Asp	Leu	Met
	1535					1540					1545			
Thr	Glu	Met	Thr	Asp	Leu	Thr	Ser	Asp	Leu	Leu	Gly	Ser	Gly	Ile
	1550					1555					1560			
Pro	Phe	Leu	Asp	Tyr	Lys	Val	Tyr	Ala	Glu	Arg	Ile	Phe	Phe	Pro
	1565					1570					1575			
Gly	His	Arg	Glu	Ser	Pro	Leu	His	Arg	Asp	Leu	Gly	Val	Pro	Glu
	1580					1585					1590			
Ser	Arg	Arg	Pro	Thr	Val	Glu	Gln	Gly	Leu	Gly	Gln	Leu	Ser	Asn
	1595					1600					1605			
Leu	Leu	Asn	Ser	Lys	Leu	Phe	Leu	Thr	Lys	Phe	Ile	His	Thr	Leu
	1610					1615					1620			
Glu	Ser	Gln	Arg	Thr	Phe	Ser	Ala	Arg	Asp	Arg	Ala	Tyr	Val	Ala
	1625					1630					1635			
Ser	Leu	Leu	Thr	Val	Ala	Leu	His	Gly	Lys	Leu	Glu	Tyr	Phe	Thr
	1640					1645					1650			
Asp	Ile	Leu	Arg	Thr	Leu	Leu	Ser	Asp	Leu	Val	Ala	Gln	Tyr	Val
	1655					1660					1665			
Ala	Lys	Asn	Pro	Lys	Leu	Met	Leu	Arg	Arg	Thr	Glu	Thr	Val	Val
	1670					1675					1680			

JPOXMLDOC01-seql.txt

Glu	Lys	Leu	Leu	Thr	Asn	Trp	Met	Ser	Ile	Cys	Leu	Tyr	Thr	Phe
	1685					1690					1695			
Val	Arg	Asp	Ser	Val	Gly	Glu	Pro	Leu	Tyr	Met	Leu	Phe	Arg	Gly
	1700					1705					1710			
Ile	Lys	His	Gln	Val	Asp	Lys	Gly	Pro	Val	Asp	Ser	Val	Thr	Gly
	1715					1720					1725			
Lys	Ala	Lys	Tyr	Thr	Leu	Asn	Asp	Asn	Arg	Leu	Leu	Arg	Glu	Asp
	1730					1735					1740			
Val	Glu	Tyr	Arg	Pro	Leu	Thr	Leu	Asn	Ala	Leu	Leu	Ala	Val	Gly
	1745					1750					1755			
Pro	Gly	Ala	Gly	Glu	Ala	Gln	Gly	Val	Pro	Val	Lys	Val	Leu	Asp
	1760					1765					1770			
Cys	Asp	Thr	Ile	Ser	Gln	Ala	Lys	Glu	Lys	Met	Leu	Asp	Gln	Leu
	1775					1780					1785			
Tyr	Lys	Gly	Val	Pro	Leu	Thr	Gln	Arg	Pro	Asp	Pro	Arg	Thr	Leu
	1790					1795					1800			
Asp	Val	Glu	Trp	Arg	Ser	Gly	Val	Ala	Gly	His	Leu	Ile	Leu	Ser
	1805					1810					1815			
Asp	Glu	Asp	Val	Thr	Ser	Glu	Val	Gln	Gly	Leu	Trp	Arg	Arg	Leu
	1820					1825					1830			
Asn	Thr	Leu	Gln	His	Tyr	Lys	Val	Pro	Asp	Gly	Ala	Thr	Val	Ala
	1835					1840					1845			
Leu	Val	Pro	Cys	Leu	Thr	Lys	His	Val	Leu	Arg	Glu	Asn	Gln	Asp
	1850					1855					1860			
Tyr	Val	Pro	Gly	Glu	Arg	Thr	Pro	Met	Leu	Glu	Asp	Val	Asp	Glu
	1865					1870					1875			
Gly	Gly	Ile	Arg	Pro	Trp	His	Leu	Val	Lys	Pro	Ser	Asp	Glu	Pro
	1880					1885					1890			
Glu	Pro	Pro	Arg	Pro	Arg	Arg	Gly	Ser	Leu	Arg	Gly	Gly	Glu	Arg
	1895					1900					1905			
Glu	Arg	Ala	Lys	Ala	Ile	Pro	Glu	Ile	Tyr	Leu	Thr	Arg	Leu	Leu
	1910					1915					1920			
Ser	Met	Lys	Gly	Thr	Leu	Gln	Lys	Phe	Val	Asp	Asp	Leu	Phe	Gln
	1925					1930					1935			

JPOXMLDOC01-seql.txt

Val Ile Leu Ser Thr Ser Arg Pro Val Pro Leu Ala Val Lys Tyr
1940 1945 1950

Phe Phe Asp Leu Leu Asp Glu Gln Ala Gln Gln His Gly Ile Ser
1955 1960 1965

Asp Gln Asp Thr Ile His Ile Trp Lys Thr Asn Ser Leu Pro Leu
1970 1975 1980

Arg Phe Trp Ile Asn Ile Ile Lys Asn Pro Gln Phe Val Phe Asp
1985 1990 1995

Val Gln Thr Ser Asp Asn Met Asp Ala Val Leu Leu Val Ile Ala
2000 2005 2010

Gln Thr Phe Met Asp Ala Cys Thr Leu Ala Asp His Lys Leu Gly
2015 2020 2025

Arg Asp Ser Pro Ile Asn Lys Leu Leu Tyr Ala Arg Asp Ile Pro
2030 2035 2040

Arg Tyr Lys Arg Met Val Glu Arg Tyr Tyr Ala Asp Ile Arg Gln
2045 2050 2055

Thr Val Pro Ala Ser Asp Gln Glu Met Asn Ser Val Leu Ala Glu
2060 2065 2070

Leu Ser Trp Asn Tyr Ser Gly Asp Leu Gly Ala Arg Val Ala Leu
2075 2080 2085

His Glu Leu Tyr Lys Tyr Ile Asn Lys Tyr Tyr Asp Gln Ile Ile
2090 2095 2100

Thr Ala Leu Glu Glu Asp Gly Thr Ala Gln Lys Met Gln Leu Gly
2105 2110 2115

Tyr Arg Leu Gln Gln Ile Ala Ala Ala Val Glu Asn Lys Val Thr
2120 2125 2130

Asp Leu
2135

<210> 5
<211> 20
<212> DNA
<213> Artificial

<220>
<223> Plxnb1 sense

<400> 5
tgggtcatgt gcagtacgat

<210> 6
 <211> 20
 <212> DNA
 <213> Artificial

<220>
 <223> Plxnb1 antisense

<400> 6
 cactgctctc caggttctcc 20

<210> 7
 <211> 20
 <212> DNA
 <213> Artificial

<220>
 <223> Plxnb2 sense

<400> 7
 aggggagcct ctctacaagc 20

<210> 8
 <211> 20
 <212> DNA
 <213> Artificial

<220>
 <223> Plxnb2 antisense

<400> 8
 tcgatccctt catcctgaac 20

<210> 9
 <211> 20
 <212> DNA
 <213> Artificial

<220>
 <223> Plxnb3 sense

<400> 9
 atatgctgag cgtgccttct 20

<210> 10
 <211> 20
 <212> DNA
 <213> Artificial

<220>
 <223> Plxnb3 antisense

<400> 10
 tgctgttgag caaattggag 20

<210> 11
 <211> 20
 <212> DNA
 <213> Artificial

<220>

<223> CD72 sense
 <400> 11
 gccttctcct gtcctgtctg 20

 <210> 12
 <211> 20
 <212> DNA
 <213> Artificial

 <220>
 <223> CD72 antisense
 <400> 12
 cctcctggaa ctgctgagac 20

 <210> 13
 <211> 20
 <212> DNA
 <213> Artificial

 <220>
 <223> Alpl sense
 <400> 13
 aacccagaca caagcattcc 20

 <210> 14
 <211> 20
 <212> DNA
 <213> Artificial

 <220>
 <223> Alpl antisense
 <400> 14
 gcctttgagg tttttggtca 20

 <210> 15
 <211> 20
 <212> DNA
 <213> Artificial

 <220>
 <223> Bglap sense
 <400> 15
 gcgctctgtc tctctgacct 20

 <210> 16
 <211> 20
 <212> DNA
 <213> Artificial

 <220>
 <223> Bglap antisense
 <400> 16
 accttattgc cctcctgctt 20

 <210> 17
 <211> 20

<212> DNA
 <213> Artificial

 <220>
 <223> Col1a1 sense

 <400> 17
 gagcggagag tactggatcg 20

 <210> 18
 <211> 20
 <212> DNA
 <213> Artificial

 <220>
 <223> Col1a1 antisense

 <400> 18
 gttcgggctg atgtaccagt 20

 <210> 19
 <211> 20
 <212> DNA
 <213> Artificial

 <220>
 <223> Gapdh sense

 <400> 19
 acccagaaga ctgtggatgg 20

 <210> 20
 <211> 20
 <212> DNA
 <213> Artificial

 <220>
 <223> Gapdh antisense

 <400> 20
 cacattgggg gtaggaacac 20