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DESCRIPTION

TECHNICAL FIELD

[0001] This invention is in the field of anti-Activin receptor IIB (ActRIIB) antibodies. In particular, it relates to compositions comprising anti-ActRIIB antibodies and use thereof for increasing brown fat in vertebrates, including rodents and primates, and particularly in humans without significantly affecting hematological parameters.

BACKGROUND OF THE INVENTION

[0002] Two types of adipose tissue can be distinguished which have very distinct features and functions: white adipose tissue (WAT) storing energy primarily as triglycerides in distinct anatomical locations and brown adipose tissue (BAT) specialized in basal and inducible energy expenditure through heat production. BAT and WAT are distinct anatomically, histologically and functionally. Until recently, brown adipose tissue (BAT) was considered of metabolic significance only in small mammals and human newborns, since it was thought to disappear rapidly after birth in humans. However, functional brown adipose tissue has been identified and characterized in adult humans promoting a renewed interest in non-shivering thermogenesis and development of future perspectives targeting BAT for pharmacological treatment of obesity and metabolic diseases.

[0003] The last decade has witnessed a profound resurgence in BAT research with a clear adult human emphasis. The need for such a dramatic increase stems from the ever-growing trend toward global human obesity. Indeed, it is currently estimated that rates of obesity in developed countries such as the United States exceed 35% of the population (Flegal, et al 2010). The higher incidence of obesity is associated with increased prevalence of the Metabolic Syndrome including diabetes, hypertension, and coronary heart disease, among others (Alberti, et al 2009, Bruce and Hanson 2010). BAT holds great promise in combating obesity given its unprecedented metabolic capacity. Although several early anatomical studies suggested that brown adipose tissue is present in adult humans (Astrup, et al 1985), its physiologic relevance was believed to be marginal. Recent controlled studies showed that functional BAT is detectable in lean, obese and morbidly obese adult humans after exposure to mild cold (Saito, et al 2009, van Marken Lichtenbelt, et al 2009, Vijgen, et al 2011, Virtanen, et al 2009). Cold induced BAT activity is inversely related to body mass index (BMI) and body fat percentage (BF%) (van Marken Lichtenbelt, et al 2009, Saito, et al 2009, Virtanen, et al 2009, Yoneshiro, et al., Obesity,Vol. 19, No.1, January 2011). Two types of brown fat cells have been reported: brown fat cells originating from a Myf5+ progenitor cells common to muscle cells and as exemplified by brown fat cells in classic locations such as interscapular, and brown fat cells interspersed in WAT which are derived from another lineage and could arise from either a dormant precursor cell or transdifferentiation from white fat (Perwitz, et al 2010, Seale, et al

2008).

The thermogenic phenotype of BAT is essentially conferred by uncoupling protein 1 (UCP1). UCP1 uncouples adenosine-5'-triphosphate (ATP) synthesis from substrate oxidation in brown adipocytes.

Brown adipose tissue (BAT) is the major site for cold- and diet-induced thermogenesis, which significantly contributes to control of the body temperature and energy expenditure, at least in small rodents such as the mouse, rat, and hamster (Cannon and Nedergaard 2004), (Klingenspor 2003), (Himms-Hagen 1990). In humans, BAT is present in newborns, but disappears rapidly during postnatal periods and, in adults, is rather difficult to identify by conventional anatomical examinations.

Brown adipose tissue has a very high uptake of glucose per gram of tissue, which means that even though the total amount of brown adipose tissue in the body is not large, it can potentially be a significant glucose-clearing organ. Physiological conditions in which plasma insulin levels are elevated show increased glucose uptake into brown adipose tissue. Brown adipose tissue is one of the most insulin-responsive tissues with respect to stimulation of glucose uptake (Cannon, Barbara, and Jan Nedergaard. Brown Adipose Tissue: Function and Physiological Significance; Physiol Rev 84: 277-359, 2004) Because of the importance of brown adipose tissue as a sink for glucose uptake, BAT manipulation opens new opportunities for the development of new therapeutics for metabolic diseases such as obesity and type-2 diabetes (Kajimura et al., Nature 460, 1154-1158., 2009).

It has been discovered that formation and/or activity of thermogenic brown adipocytes was increased in white fat tissue of mice treated with an ActRIIB antagonists (WO2010144452 or US201310577). However, it has also been discovered that treatment with ActRIIB antagonists, for example, soluble ActRIIB proteins causes statistically significant hematological changes, including elevated reticulocyte levels and red blood cell distribution width values. Excessive increases in red blood cell levels or cell width, hemoglobin levels, or hematocrit levels may (i) cause increases in blood pressure, (ii) slow down blood velocity, and (iii) increase the risk of sludging, thrombosis or stroke. Consequently, it has been recommended to restrict dosing of ActRIIB antagonists to patients who have appropriate hematologic parameters or patients that have both anemia and muscle loss (WO/2009/158025). Additionally, a method of managing patients treated with an ActRIIB antagonist, comprising monitoring hematologic parameters that correlate with increased red blood cell levels has been developed (WO2009/158025). Consequently, there is a need to develop novel methods for the treatment of metabolic disorders like Metabolic Syndrome, obesity, insulin resistance, Type 2 Diabetes mellitus and/or increasing brown adipose tissue (BAT) in a patient while not inducing significant hematological changes in said subject.

SUMMARY OF THE INVENTION

[0004] There is a need to develop novel pharmaceutical compositions and methods for the treatment of metabolic disorders like Metabolic Syndrome, obesity, insulin resistance, Type 2 Diabetes mellitus and/or increasing brown adipose tissue (BAT) in a patient while not inducing significant hematological changes in said subject. This objective is achieved by the methods

and compositions provided within this disclosure.

[0005] A first subject matter of the disclosure therefore relates to a composition comprising an antagonist antibody binding to ActRIIB for use in treating a metabolic disorder in a subject, wherein the anti-ActRIIB antibody increases brown adipose tissue without increasing red blood cell levels in said subject and wherein the anti-ActRIIB antibody binds to a binding domain consisting of amino acids 19-134 of SEQ ID NO: 181 (SEQ ID NO: 182); and wherein the metabolic disorder is selected from the group consisting of obesity, Type 2 Diabetes, Metabolic Syndrome, lipodystrophy, impaired glucose tolerance, elevated plasma insulin concentration, insulin resistance, dyslipidemia, hyperglycemia, hyperlipidemia, hypertension, cardiovascular disease and respiratory conditions.

[0006] . The disclosed compositions are also suited to treat a subject suffering from a metabolic and a muscle disorder. In one embodiment the muscle disorder is a muscle atrophy selected from the group consisting of obesity-associated sarcopenia, sarcopenia, or diabetes-associated muscle atrophy

[0007] In another embodiment the composition comprises an anti-ActRIIB antibody which binds to a binding domain consisting of amino acids 19-134 of SEQ ID NO: 181 (SEQ ID NO:182), or to an epitope comprising or consisting of (a) amino acids 78-83 of SEQ ID NO: 181 (WLDDFN - SEQ ID NO:188); (b) amino acids 76-84 of SEQ ID NO:181 (GCWLDDFNC - SEQ ID NO:186); (c) amino acids 75-85 of SEQ ID NO:181 (KGCWLDDFNCY - SEQ ID NO:190); (d) amino acids 52-56 of SEQ ID NO:181 (EQDKR - SEQ ID NO:189); (e) amino acids 49-63 of SEQ ID NO:181 (CEGEQDKRLHCYASW - SEQ ID NO:187); (f) amino acids 29-41 of SEQ ID NO:181 (CIYYNANWELERT- SEQ ID NO:191); (g) amino acids 100-110 of SEQ ID NO:181 (YFCCCEGNFCN - SEQ ID NO:192); or (h) amino acids 78-83 of SEQ ID NO:181 (WLDDFN) and amino acids 52-56 of SEQ ID NO:181 (EQDKR).

[0008] In yet another alternative embodiment the above mentioned compositions comprise an anti-ActRIIB antibody which binds ActRIIB with a 10-fold or greater affinity than it binds to ActRIIA.

[0009] Additionally, the disclosure relates to composition wherein the anti-ActRIIB antibody comprises a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14; a heavy chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15-28; a heavy chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 29-42; a light chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 43-56; a light chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 57-70; and a light chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 71-84.
In certain embodiments, the disclosure provides compositions wherein the anti-ActRIIB antibody comprises: (a) a heavy chain variable region CDR1 of SEQ ID NO: 1; a heavy chain

variable region CDR2 of SEQ ID NO: 15; a heavy chain variable region CDR3 of SEQ ID NO: 29; a light chain variable region CDR1 of SEQ ID NO: 43; a light chain variable region CDR2 of SEQ ID NO: 57; and a light chain variable region CDR3 of SEQ ID NO: 71, (b) a heavy chain variable region CDR1 of SEQ ID NO: 2; a heavy chain variable region CDR2 of SEQ ID NO: 16; a heavy chain variable region CDR3 of SEQ ID NO: 30; a light chain variable region CDR1 of SEQ ID NO: 44; a light chain variable region CDR2 of SEQ ID NO: 58; and a light chain variable region CDR3 of SEQ ID NO: 72, (c) a heavy chain variable region CDR1 of SEQ ID NO: 3; a heavy chain variable region CDR2 of SEQ ID NO: 17; a heavy chain variable region CDR3 of SEQ ID NO: 31; a light chain variable region CDR1 of SEQ ID NO: 45; a light chain variable region CDR2 of SEQ ID NO: 59; and a light chain variable region CDR3 of SEQ ID NO: 73, (d) a heavy chain variable region CDR1 of SEQ ID NO: 4; a heavy chain variable region CDR2 of SEQ ID NO: 18; a heavy chain variable region CDR3 of SEQ ID NO: 32; a light chain variable region CDR1 of SEQ ID NO: 46; a light chain variable region CDR2 of SEQ ID NO: 60; and a light chain variable region CDR3 of SEQ ID NO: 74, (e) a heavy chain variable region CDR1 of SEQ ID NO: 5; a heavy chain variable region CDR2 of SEQ ID NO: 19; a heavy chain variable region CDR3 of SEQ ID NO: 33; a light chain variable region CDR1 of SEQ ID NO: 47; a light chain variable region CDR2 of SEQ ID NO: 61; and a light chain variable region CDR3 of SEQ ID NO: 75, (f) a heavy chain variable region CDR1 of SEQ ID NO: 6; a heavy chain variable region CDR2 of SEQ ID NO: 20; a heavy chain variable region CDR3 of SEQ ID NO: 34; a light chain variable region CDR1 of SEQ ID NO: 48; a light chain variable region CDR2 of SEQ ID NO: 62; and a light chain variable region CDR3 of SEQ ID NO: 76, (g) a heavy chain variable region CDR1 of SEQ ID NO: 7; a heavy chain variable region CDR2 of SEQ ID NO: 21; a heavy chain variable region CDR3 of SEQ ID NO: 35; a light chain variable region CDR1 of SEQ ID NO: 49; a light chain variable region CDR2 of SEQ ID NO: 63; and a light chain variable region CDR3 of SEQ ID NO: 77, (h) a heavy chain variable region CDR1 of SEQ ID NO: 8; a heavy chain variable region CDR2 of SEQ ID NO: 22; a heavy chain variable region CDR3 of SEQ ID NO: 36; a light chain variable region CDR1 of SEQ ID NO: 50 a light chain variable region CDR2 of SEQ ID NO: 64; and a light chain variable region CDR3 of SEQ ID NO: 78, (i) a heavy chain variable region CDR1 of SEQ ID NO: 9; a heavy chain variable region CDR2 of SEQ ID NO: 23; a heavy chain variable region CDR3 of SEQ ID NO: 37; a light chain variable region CDR1 of SEQ ID NO: 51; a light chain variable region CDR2 of SEQ ID NO: 65; and a light chain variable region CDR3 of SEQ ID NO: 79, (j) a heavy chain variable region CDR1 of SEQ ID NO: 10; a heavy chain variable region CDR2 of SEQ ID NO: 24; a heavy chain variable region CDR3 of SEQ ID NO: 38; a light chain variable region CDR1 of SEQ ID NO: 52; a light chain variable region CDR2 of SEQ ID NO: 66; and a light chain variable region CDR3 of SEQ ID NO: 80, (k) a heavy chain variable region CDR1 of SEQ ID NO: 11; a heavy chain variable region CDR2 of SEQ ID NO: 25; a heavy chain variable region CDR3 of SEQ ID NO: 39; a light chain variable region CDR1 of SEQ ID NO: 53; a light chain variable region CDR2 of SEQ ID NO: 67; and a light chain variable region CDR3 of SEQ ID NO: 81, (l) a heavy chain variable region CDR1 of SEQ ID NO: 12; a heavy chain variable region CDR2 of SEQ ID NO: 26; a heavy chain variable region CDR3 of SEQ ID NO: 40; a light chain variable region CDR1 of SEQ ID NO: 54; a light chain variable region CDR2 of SEQ ID NO: 68; and a light chain variable region CDR3 of SEQ ID NO: 82, (m) a heavy chain variable region CDR1 of SEQ ID NO: 13; a heavy chain variable region CDR2 of SEQ ID NO: 27; a heavy chain variable

region CDR3 of SEQ ID NO: 41; a light chain variable region CDR1 of SEQ ID NO: 55; a light chain variable region CDR2 of SEQ ID NO: 69; and a light chain variable region CDR3 of SEQ ID NO: 83, or (n) a heavy chain variable region CDR1 of SEQ ID NO: 14; a heavy chain variable region CDR2 of SEQ ID NO: 28; a heavy chain variable region CDR3 of SEQ ID NO: 42; a light chain variable region CDR1 of SEQ ID NO: 56; a light chain variable region CDR2 of SEQ ID NO: 70; and a light chain variable region CDR3 of SEQ ID NO: 84.

[0010] In yet another embodiment, the above mentioned anti-ActRIIB antibody comprises (i) a full length heavy chain amino acid sequence having at least 95% sequence identity to at least one sequence selected from the group consisting of SEQ ID NOs:146-150 and 156-160, (ii) a full length light chain amino acid sequence having at least 95% sequence identity to at least one sequence selected from the group consisting of SEQ ID NOs:141-145 and 151-155 or (iii) (a) the variable heavy chain sequence of SEQ ID NO: 99 and variable light chain sequence of SEQ ID NO: 85; (b) the variable heavy chain sequence of SEQ ID NO: 100 and variable light chain sequence of SEQ ID NO: 86; (c) the variable heavy chain sequence of SEQ ID NO: 101 and variable light chain sequence of SEQ ID NO: 87; (d) the variable heavy chain sequence of SEQ ID NO: 102 and variable light chain sequence of SEQ ID NO: 88; (e) the variable heavy chain sequence of SEQ ID NO: 103 and variable light chain sequence of SEQ ID NO: 89; (f) the variable heavy chain sequence of SEQ ID NO: 104 and variable light chain sequence of SEQ ID NO: 90; (g) the variable heavy chain sequence of SEQ ID NO: 105 and variable light chain sequence of SEQ ID NO: 91; (h) the variable heavy chain sequence of SEQ ID NO: 106 and variable light chain sequence of SEQ ID NO: 92; (i) the variable heavy chain sequence of SEQ ID NO: 107 and variable light chain sequence of SEQ ID NO: 93; (j) the variable heavy chain sequence of SEQ ID NO: 108 and variable light chain sequence of SEQ ID NO: 94; (k) the variable heavy chain sequence of SEQ ID NO: 109 and variable light chain sequence of SEQ ID NO: 95; (l) the variable heavy chain sequence of SEQ ID NO: 110 and variable light chain sequence of SEQ ID NO: 96; (m) the variable heavy chain sequence of SEQ ID NO: 111 and variable light chain sequence of SEQ ID NO: 97; or (n) the variable heavy chain sequence of SEQ ID NO: 112 and variable light chain sequence of SEQ ID NO: 98.
In certain aspects the disclosure relates to the above described compositions, wherein the comprised anti-ActRIIB antibody comprises (a) the heavy chain sequence of SEQ ID NO: 146 and light chain sequence of SEQ ID NO: 141; (b) the heavy chain sequence of SEQ ID NO: 147 and light chain sequence of SEQ ID NO: 142; (c) the heavy chain sequence of SEQ ID NO: 148 and light chain sequence of SEQ ID NO: 143; (d) the heavy chain sequence of SEQ ID NO: 149 and light chain sequence of SEQ ID NO: 144; (e) the heavy chain sequence of SEQ ID NO: 150 and light chain sequence of SEQ ID NO: 145; (f) the heavy chain sequence of SEQ ID NO: 156 and light chain sequence of SEQ ID NO: 151; (g) the heavy chain sequence of SEQ ID NO: 157 and light chain sequence of SEQ ID NO: 152; (h) the heavy chain sequence of SEQ ID NO: 158 and light chain sequence of SEQ ID NO: 153; (i) the heavy chain sequence of SEQ ID NO: 159 and light chain sequence of SEQ ID NO: 154; or (j) the heavy chain sequence of SEQ ID NO: 160 and light chain sequence of SEQ ID NO: 155.

[0011] An additional subject matter of the disclosure relates to composition, wherein (i) the anti-ActRIIB antibody cross-blocks or is cross blocked by one of the above described

antibodies, (ii) has altered effector function through mutation of the Fc region and/or (iii) binds to an epitope recognized by one of the above described antibodies.

[0012] In yet another embodiment, the disclosed composition comprises an anti-ActRIIB antibody encoded by pBW522 (DSM22873) or pBW524 (DSM22874).

[0013] Furthermore, the disclosure provides a method for decreasing the average plasma glucose concentration in a subject while not increasing red blood cell levels and/or without resulting in a clinically unacceptable change in hematocrit or other blood parameter level, comprising the step of administering to said subject an antibody binding to ActRIIB. In certain aspects, the average plasma glucose concentration is detected by measuring the glycated hemoglobin (glycosylated hemoglobin) levels.

[0014] In another aspect the disclosure provides a method of treating a metabolic disorder, like those described above, in a subject, wherein the subject is suffering from a metabolic disorder and a muscle disorder. In another embodiment, the above mentioned muscle disorder is a muscle atrophy selected from the group consisting of obesity-associated sarcopenia, sarcopenia, or diabetes-associated muscle atrophy.

BRIEF DESCRIPTION OF THE FIGURES

[0015]

Figure 1: The activin receptor IIB inhibits the differentiation of primary brown adipocytes. Primary brown pre-adipocytes isolated from mouse inter-scapular brown adipose tissue were differentiated during 9 days using a classical adipogenic medium (AM) containing insulin and thyroid hormone for the entire protocol and IBMX, 3-isobutyl-1-methylxanthine, dexamethasone and indomethacin for the first two days. Adipogenic medium (AM), AM + myostatin (Mstn; 10ng/ml), a monoclonal antibody against the activin receptor IIB (AM + MOR08159 (Antibody comprising heavy chain sequence of SEQ ID NO: 146 and light chain sequence of SEQ ID NO: 141); 10µg/ml) or a combination of both (AM+ MOR08159 + Mstn); was added to the adipogenic medium and fresh medium and treatments were replaced every 2 days. The level of differentiation was evaluated by measuring the mRNA expression of classical brown fat markers like UCP1 from 2 independent cultures for each condition (PRDM16: PR domain containing 16, PGC-1a/b: peroxisome proliferative activated receptor, gamma, coactivator 1 alpha).

Figure 2: ActRIIB inhibition in mice increases the amount of brown but not white fat. SCID (*s*evere *c*ombined *i*mmuno *d*eficiency) mice (n=12/group) were treated for 4 weeks with a weekly sub-cutaneous injection of control antibody (IgG1; 20mg/kg/week) or increasing amounts of a human monoclonal antibody against ActRIIB (MOR08159; 2, 6 and 20 mg/kg/week). The wet mass of gastrocnemius muscle, inter-scapular brown adipose tissue (BAT) and epididymal white adipose tissue (WAT) was measured and expressed as a

percentage change from the average of the control group

Figure 3: ActRIIB inhibition increases cold tolerance. Cold tolerance was evaluated by placing C57BL6/J mice (n=11/group) that had been treated for 3 weeks with a weekly sub-cutaneous injection of vehicle or a mouse variant of the monoclonal antibody against ActRIIB (Chimeric antibody comprising heavy chain sequence of SEQ ID NO: 194 and light chain sequence of SEQ ID NO: 193, 20 mg/kg/week) at 10°C for 4h and measuring rectal body temperature every hour.

Figure 4: ActRIIB inhibition increases cellular respiration of brown fat. Primary brown adipocytes were differentiated and treated as described in figure 1 (n=10-20 per condition) and the oxygen consumption rate was measured under basal conditions or in the presence of 2.5µg/ml oligomycin (uncoupled) or 500nM final FCCP (carbonylcyanide-p-(trifluoromethoxy)-phenylhydrazone,) (maximal).

GENERAL DEFINITIONS

[0016] In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.
The term "comprising" means "including" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

[0017] The term "about" in relation to a numerical value x means, for example, *x*±10%.

[0018] The term "hematological changes" refer to changes in hematologic parameters like red blood cell levels (RBC: red blood cells absolute count), red blood cell distribution width (RDW) or mean corpuscular hemoglobin concentration (MCHC). In a preferred embodiment, the term refers to an increase of at least one of the above mentioned parameters. In other words, the "hematological changes" in the context of the disclosed teaching refers to the fact that treatment of a subject with the disclosed compositions does not lead to a statistically significant increase in RBC, RDW and/or MCHC in said subject. RBC, RDW and/or MCHC values can be determined using art recognized methods. Physiologically normal red blood cell count is known to the skilled person. RBC levels may be determined by counting red blood cells (using commercially available Coulter Counter), by hemoglobin or hematocrit level measurements. Hematocrit (Hct) or packed cell volume (PCV) refers to the ratio of the volume of red blood cells to the volume of whole blood. Hematocrit may be determined, for example, by centrifugation of a blood sample followed by analysis of the layers produced. Physiological normal ranges for hematocrit for men and for women are known to the skilled person.
Hematocrit levels within the human or mammalian animal body are primarily a percentage of the red blood cells in a certain amount of plasma. For instance, when a blood test has been conducted, the readings will show on the blood test report as HCT levels at 40%. This is indicative of the fact that for every 100 milliliters of blood in the patient's body, about 40

milliliters of it is primarily red blood cells. Hemoglobin levels can be determined by lysing red blood cells and conversion of the hemoglobin into cyanomethemoglobin and measurement of hemoglobin with a colorimeter. Physiologically normal levels of hemoglobin adult males and females are known to the skilled person.

[0019] The phrase "not increasing red blood cell levels" or "without increasing red blood cell levels" in a subject refer to a condition in which the red blood cell- (e.g RBC), hematocrit- or hemoglobin-level is not significantly increased compared to the red blood cell-, hematocrit-, or hemoglobin-level in said patient prior to a single or multiple dose treatment up to three months after treatment start. Consequently, an increase in red blood cell levels would result in clinically unacceptable changes in hematocrit or other blood parameter level in a subject.

[0020] "Not increasing red blood cell levels" or "without increasing red blood cell levels" in a subject with acceptable or normal red blood levels also refers to a condition in which the red blood cell-, hematocrit- or hemoglobin-levels do not increase beyond the accepted range or normal. Normal hematocrit levels in children at the age of about 10 years will be between about 36 and about 40% while adult females will show readings of between about 36% and about 46% with adult males reflecting the readings between about 41 and about 54%. Any readings outside these normal ranges, provided that the values of the treated individual prior to the treatment were not above said ranges, will be considered as a condition in which red blood cells levels are increased. Hemoglobin level is expressed as the amount of hemoglobin in grams (gm) per deciliter (dl) of whole blood, a deciliter being 100 milliliters. The normal ranges for hemoglobin depend on the age and, beginning in adolescence, the gender of the person. The normal ranges are: children: about 11-13 gm/dl, adult males: about 14-18 gm/dl, adult women: about 12-16 gm/dl, men after middle age: about 12.4-14.9 gm/dl, women after middle age: about 11.7-13.8 gm/dl. Any hemoglobin levels outside these normal ranges, provided that the values of the treated individual prior to the treatment were not above said ranges, will be considered as a condition in which red blood cells levels are increased. The phrase "not increasing red blood cell levels" or "without increasing red blood cell levels" in a subject refers to a condition were an increase in hematocrit from the normal range to beyond the accepted range of normal is avoided.
The following exemplifies a possible pre-clinical treatment regime to evaluate possible effects of a treatment with an ActRIIB antibody. The treatment is exemplified by using cynomolgus monkeys, but the described experiments are not limited to monkeys and the skilled person knows how to set up suitable experiments or dosing regimens for other species, in particular for humans: the ActRIIB antibody can be administered once a week for 3 months to male and female cynomolgus monkeys by intravenous injection. 32 cynomolgus monkeys (16/sex) can be assigned to one of four treatment groups (3 to 5 animals/sex/group) and can be administered intravenous injections of either vehicle or the ActRIIB antibody at 10, 30, or 100 mg/kg once weekly for 13 weeks (total of 14 doses; doses shall be selected on the basis of muscle hypertrophy activity in monkey).

[0021] The phrase "increased average plasma glucose concentration" refers to a physiological situation in a patient in which the plasma glucose concentration (blood sugar concentration or

blood glucose level or presence of glucose in the blood of a human or animal) would be considered by a skilled person as being outside of the normal range of a healthy individual. Blood glucose levels are measured in molar concentration (millimoles per litre; or millimolar, abbreviated mM). The skilled person is aware of normal blood glucose levels in mammals, which are tightly regulated as a part of metabolic homeostasis. However, blood glucose levels fluctuate throughout the day (lowest in the morning and rise after meals).

[0022] Glycated hemoglobin (glycosylated hemoglobin, hemoglobin A1c, HbA1c, A1C, or Hb1c; sometimes also HbA1c) is a form of hemoglobin which is measured primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. Hence, glycated hemoglobin serves as a marker for average blood glucose levels. Methods for determining the glycated hemoglobin level -as dependable methods of assessing glycemic control- are well know in the art (H.B. Chandalia and P.R. Krishnaswamy, Current Science, Vol. 83, No. 12, 25 December 2002)

[0023] The phrase "increases brown adipose tissue" or "increase in brown adipose tissue (BAT)" refers to (i) an increase in wet mass of inter-scapular brown adipose tissue (BAT) measured and expressed as a percentage change from the average of a defined control group, wherein the increase is statistically significant and is influenced by the amount of administered ActRIIB antibody and compositions comprising the same; or (ii) an enhancement in the differentiation of primary brown adipocytes as reflected by an increase in expression of thermogenic genes such as UCP-1. Moreover, brown adipose tissue can be analysed using positron-emission tomography and computed tomography (Cypess et al., 2009, N Engl J Med 360:1509- 1517).

[0024] The term "brown adipose tissue" in the context of this invention should be understood as also referring to or covering the terms "brown fat" "brown adipocytes" and "thermogenic adipocytes", which are used interchangeably.

[0025] The term "metabolic disorder" as used in context of this invention refers to a disease or condition that is impacted by the presence, level or activity of brown adipose tissue, plasma glucose concentration, plasma insulin level and/or body fat content. A metabolic disorder or condition also includes, but is not limited to, Metabolic Syndrome, impaired glucose tolerance, elevated plasma insulin concentrations and insulin resistance, dyslipidemia, hyperglycemia, hyperlipidemia, hypertension, lipodystrophy, cardiovascular disease, respiratory problems or conditions. Metabolic disorders of particular interest are type 2 diabetes mellitus (also known as non- insulin-dependent diabetes mellitus or adult-onset diabetes), which is characterized by elevated blood glucose in the context of insulin resistance and relative insulin deficiency, obesity, cardiovascular disease, stroke, respiratory problems. Type 2 Diabetes, or insulin-dependent diabetes (i.e., non- insulin-dependent diabetes mellitus), often occurs in the face of normal, or even elevated, levels of insulin and appears to be the result of the inability of tissues

to respond appropriately to insulin. Most type II diabetics are also obese.

[0026] The term "obesity" refers to a physiological condition of an individual in which there is an excess of body fat diagnosed by a physician. Obesity can be calculated using the body mass index (BMI: body weight per height in meters squared). According to this system, obesity is defined as an otherwise healthy subject that has a BMI greater than or equal to 30 kg/m2, or a condition whereby a subject with at least one co-morbidity has a BMI greater than or equal to 27 kg/m2.

[0027] The terms "ActRIIA" and "ActRIIB" refer to Activin receptors. Activins signal through a heterodimeric complex of receptor serine kinases which include at least two type I (I and IB) and two type II (II and IIB, aka ACVR2A and ACVR2B) receptors. These receptors are all transmembrane proteins, composed of a ligand-binding extracellular domain with a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine specificity. Type I receptors are essential for signaling while type II receptors are required for binding ligands and for expression/recruitment of type I receptors. Type I and II receptors form a stable complex after ligand binding resulting in the phosphorylation of type I receptors by type II receptors. The activin receptor II B (ActRIIB) is a receptor for myostatin.

[0028] The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (e.g. antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the human body of invading pathogens, cells or tissues infected with pathogens, cancerous cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.
A "signaling activity" refers to a biochemical causal relationship generally initiated by a protein-protein interaction such as binding of a growth factor to a receptor, resulting in transmission of a signal from one portion of a cell to another portion of a cell. In general, the transmission involves specific phosphorylation of one or more tyrosine, serine, or threonine residues on one or more proteins in the series of reactions causing signal transduction. Penultimate processes typically include nuclear events, resulting in a change in gene expression.
The term ActRIIB or Act IIB receptor refers to human ActRIIB as defined in SEQ ID NO: 181 (AAC64515.1, GI:3769443). Research grade polyclonal and monoclonal anti-ActRIIB antibodies are known in the art, such as those made by R&D Systems®, MN, USA. Of course, antibodies could be raised against ActRIIB from other species and used to treat pathological conditions in those species.

[0029] The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (*i.e.* "antigen-binding portion") or single chains thereof. A naturally occurring "antibody" is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant

region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.* effector cells) and the first component (Clq) of the classical complement system.
The term "antigen-binding portion" of an antibody (or simply "antigen portion"), as used herein, refers to full length or one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.* a portion of ActRIIB). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and CH1 domains; a $F(ab)_2$ fragment, a bivalent fragment comprising two Fab fragments, each of which binds to the same antigen, linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the V_H and CH1 domains; a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; a dAb fragment (Ward et al., 1989 Nature 341:544-546), which consists of a V_H domain; and an isolated complementarity determining region (CDR).
Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.* Bird et al., 1988 Science 242:423-426; and Huston et al., 1988 Proc. Natl. Acad. Sci. 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding region" of an antibody. These antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[0030] An "isolated antibody", as used herein, refers to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.* an isolated antibody that specifically binds ActRIIB is substantially free of antibodies that specifically bind antigens other than ActRIIB). An isolated antibody that specifically binds ActRIIB may, however, have cross-reactivity to other antigens, such as ActRIIB molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0031] The terms "cross-block", "cross-blocked" and "cross-blocking" are used interchangeably herein to mean the ability of an antibody or other binding agent to interfere with the binding of other antibodies or binding agents to ActRIIB, particularly the ligand binding domain, in a standard competitive binding assay.
The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody

composition displays a single binding specificity and affinity for a particular epitope.

[0032] The term "human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody contains a constant region, the constant region also is derived from such human sequences, *e.g.* human germline sequences, or mutated versions of human germline sequences or antibody containing consensus framework sequences derived from human framework sequences analysis, for example, as described in Knappik, et al. (2000. J Mol Biol 296, 57-86).
The human antibodies of the invention may include amino acid residues not encoded by human sequences (*e.g.* mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0033] The term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable regions in which both the framework and CDR regions are derived from human sequences. In one embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, *e.g.* a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.
The term "recombinant human antibody", as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (*e.g.* a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed to express the human antibody, *e.g.* from a transfectoma, antibodies isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of all or a portion of a human immunoglobulin gene, sequences to other DNA sequences. Such recombinant human antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0034] As used herein, "isotype" refers to the antibody class (*e.g.* IgM, IgE, IgG such as IgG1 or IgG2) that is provided by the heavy chain constant region genes.
The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen".

[0035] As used herein, an antibody that "specifically binds to ActRIIB polypeptide" is intended to refer to an antibody that binds to human ActRIIB polypeptide with a K_D of a 100nM or less,

10nM or less, 1nM or less. An antibody that "cross-reacts with an antigen other than ActRIIB" is intended to refer to an antibody that binds that antigen with a K_D of 10 x 10^{-9} M or less, 5 x 10^{-9} M or less, or 2 x 10^{-9} M or less. An antibody that "does not cross-react with a particular antigen" is intended to refer to an antibody that binds to that antigen, with a K_D of 1.5 x 10^{-8} M or greater, or a K_D of 5-10 x 10^{-8} M, or 1 x 10^{-7} M or greater. In certain embodiments, such antibodies that do not cross-react with the antigen exhibit essentially undetectable binding against these proteins in standard binding assays. K_D may be determined using a biosensor system, such as a Biacore® system, or Solution Equilibrium Titration.

[0036] As used herein, the term "antagonist antibody" is intended to refer to an antibody that inhibits ActRIIB induced signaling activity in the presence of myostatin or of other lActRIIB ligands such as activins or GDF-11. Examples of an assay to detect this include inhibition of myostatin induced signalling (for instance by a Smad dependent reporter gene assay), inhibition of myostatin induced Smad phosphorylation (P-Smad ELISA) and inhibition of myostatin induced inhibition of skeletal muscle cell differentiation (for instance by a creatine kinase assay).

[0037] In some embodiments, the antibodies inhibit myostatin induced signalling as measured in a Smad dependent reporter gene assay at an IC50 of 10nM or less, 1nM or less, or 100pM or less.

[0038] As used herein, an antibody with "no agonistic activity" is intended to refer to an antibody that does not significantly increase ActRIIB mediated signaling activity in the absence of myostatin in a cell-based assay, such as inihibition of myostatin induced signalling (for instance by a Smad dependent reporter gene assay), inhibition of myostatin induced Smad phosphorylation (P-Smad ELISA) and inhibition of myostatin induced inhibition of skeletal muscle cell differentiation (for instance by a creatine kinase assay). Such assays are described in more details in the examples below.

[0039] The term "K_{assoc}" or "K_a", as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "K_{dis}" or "K_d", as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "K_D", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (*i.e.* K_d/K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A method for determining the K_D of an antibody is by using surface plasmon resonance, such as the biosensor system of Biacore®, or Solution Equilibrium Titration (SET) (see Friguet B et al. (1985) J. Immunol Methods; 77(2): 305-319, and Hanel C et al. (2005) Anal Biochem; 339(1): 182-184).

[0040] As used herein, the term "Affinity" refers to the strength of interaction between antibody and antigen at single antigenic sites. Within each antigenic site, the variable region of the

antibody "arm" interacts through weak non-covalent forces with antigen at numerous sites; the more interactions, the stronger the affinity.

[0041] As used herein, the term "Avidity" refers to an informative measure of the overall stability or strength of the antibody-antigen complex. It is controlled by three major factors: antibody epitope affinity; the valency of both the antigen and antibody; and the structural arrangement of the interacting parts. Ultimately these factors define the specificity of the antibody, that is, the likelihood that the particular antibody is binding to a precise antigen epitope.

[0042] As used herein, the term "ADCC" or "antibody dependent cellular cytotoxicity" activity refers to human B cell depleting activity. ADCC activity can be measured by the human B cell depleting assays known in the art.
In order to get a higher avidity probe, a dimeric conjugate (two molecules of an antibody protein coupled to a FACS marker) can be constructed, thus making low affinity interactions (such as with the germline antibody) more readily detected by FACS. In addition, another means to increase the avidity of antigen binding involves generating dimers, trimers or multimers of any of the constructs described herein of the anti-ActRIIB antibodies. Such multimers may be generated through covalent binding between individual modules, for example, by imitating the natural C-to-N-terminus binding or by imitating antibody dimers that are held together through their constant regions. The bonds engineered into the Fc/Fc interface may be covalent or non-covalent. In addition, dimerizing or multimerizing partners other than Fc can be used in ActRIIB hybrids to create such higher order structures. For example, it is possible to use multimerizing domains such as the trimerizing domain described in WO2004/039841 or pentamerizing domain described in WO98/18943.

[0043] As used herein, the term "selectivity" for an antibody refers to an antibody that binds to a certain target polypeptide but not to closely related polypeptides.
As used herein, the term "high affinity" for an antibody refers to an antibody having a K_D of 1nM or less for a target antigen. As used herein, the term "subject" includes any human or nonhuman animal.

[0044] The term "nonhuman animal" includes all vertebrates, *e.g.* mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, *etc.*

[0045] As used herein, the term, "optimized" means that a nucleotide sequence has been altered to encode an amino acid sequence using codons that are preferred in the production cell or organism, generally a eukaryotic cell, for example, a cell of *Pichia,* a cell of *Trichoderma,* a Chinese Hamster Ovary cell (CHO) or a human cell. The optimized nucleotide sequence is engineered to retain completely or as much as possible the amino acid sequence originally encoded by the starting nucleotide sequence, which is also known as the "parental" sequence. The optimized sequences herein have been engineered to have codons that are preferred in CHO mammalian cells, however optimized expression of these sequences in other eukaryotic

cells is also envisioned herein. The amino acid sequences encoded by optimized nucleotide sequences are also referred to as optimized.

DETAILED DESCRIPTION OF THE INVENTION

[0046] It has been discovered that antibodies directed to the ActRIIB receptor can prevent myostatin from binding to the receptor, thus influencing the glucose homeostasis and/or fat metabolism in a subject without negatively impacting hematological parameters in said subject. This leads to metabolic or physiological effects like an increase in brown adipose tissue, improved glucose homeostasis, normalization of insulin levels, decrease in average plasma glucose concentration and/or decrease in glycosylated hemoglobin in a patient/subject. One further advantage of the disclosed teaching resides in the fact that it is not necessary to monitor hematologic parameters prior, during or after the administration of the compositions of the invention. Furthermore, compared to the prior art methods, there is no need to restrict the treatment of the disclosed compositions to patients/subjects who have appropriate hematologic parameters. For example, patients/subjects who have a red blood cell-, hemoglobin or hematorcrit-level above normal can also be treated. Therefore, in one aspect, the invention provides a composition comprising an antagonist antibody binding to ActRIIB for use in treating a metabolic disorder in a subject, wherein the anti-ActRIIB antibody increases brown adipose tissue without increasing red blood cell levels in said subject and wherein the anti-ActRIIB antibody binds to a binding domain consisting of amino acids 19-134 of SEQ ID NO: 181 (SEQ ID NO: 182); and wherein the metabolic disorder is selected from the group consisting of obesity, Type 2 Diabetes, Metabolic Syndrome, lipodystrophy, impaired glucose tolerance, elevated plasma insulin concentration, insulin resistance, dyslipidemia, hyperglycemia, hyperlipidemia, hypertension, cardiovascular disease and respiratory conditions.

[0047] In one embodiment, the antibodies comprised in the disclosed composition bind to ActRIIB with a K_D of 100nM or less, 10nM or less, 1 nM or less. Preferably, the antibodies comprised in the disclosed composition bind to ActRIIB with an affinity of 100pM or less (*i.e.* 100pM, 50pM, 10pM, 1pM or less). In one embodiment, the antibodies comprised in the disclosed composition bind to ActRIIB with an affinity of between 10 and 20pM.
In one embodiment, the antibodies comprised in the disclosed composition do not cross-react with an ActRIIB related protein, particularly do not cross-react with human ActRIIA (NP_001607.1, GI:4501897), more particularly, the antibodies comprised in the disclosed composition bind to ActRIIB with 5-fold greater affinity than they bind to ActRIIA, more preferably 10-fold, still more preferably 50-fold, still more preferably 100-fold.

[0048] In one embodiment, the antibodies comprised in the disclosed composition bind to ActRIIA with an affinity of 100pM or more (*i.e.*250pM, 500pM, 1nM, 5nM or more).
In one embodiment the antibodies comprised in the disclosed composition are of the IgG2 isotype.
In another embodiment, the antibodies comprised in the disclosed composition are of the IgG1 isotype. In a further embodiment, the antibodies comprised in the disclosed composition are of

the IgG1 isotype and have an altered effector function through mutation of the Fc region. Said altered effector function may be a reduced ADCC and CDC activity. In one embodiment, said altered effector function is silenced ADCC and CDC activity.
In another related embodiment, the antibodies comprised in the disclosed composition are fully human or humanized IgG1 antibodies with no antibody dependent cellular cytotoxicity (ADCC) activity or CDC activity and bind to a region of ActRIIB consisting of amino acids 19-134 of SEQ ID NO:181.
In another related embodiment, the antibodies comprised in the disclosed composition are fully human or humanized IgG1 antibodies with reduced antibody dependent cellular cytotoxicity (ADCC) activity or CDC activity and bind to a region of ActRIIB consisting of amino acids 19-134 of SEQ ID NO:181.
The present disclosure relates to compositions comprising human or humanized ActRIIB antibodies for use in treating a subject to increase brown adipose tissue while not increasing red blood cell levels.
. Application of the disclosed compositions leads to a reduction of average plasma glucose concentration, normalization of glucose homeostasis and/or plasma insulin concentrations while not increasing red blood cell levels in said subject. Average plasma glucose concentration can be measured by determining the level of glycated hemoglobin (glycosylated hemoglobin, hemoglobin A1c, HbA1c, A1C, Hb1c or HbA1c; H.B. Chandalia and P.R. Krishnaswamy, Current Science, Vol. 83, No. 12, 25 December 2002)

[0049] In an alternative embodiment the disclosure relates to compositions comprising an antibody binding to ActRIIB for use according to the present claims in treating a subject for decreasing the average plasma glucose concentration in a subject, while not increasing red blood cell levels.

[0050] In another embodiment, the patient treated with the disclosed compositions suffers from a metabolic or physiological and a muscle disorder. There are many causes of muscle disordes (e.g. atrophy), including as a result of treatment with a glucocorticoid such as cortisol, dexamethasone, betamethasone, prednisone, methylprednisolone, or prednisolone. The muscle atrophy can also be a result of denervation due to nerve trauma or a result of degenerative, metabolic, or inflammatory neuropathy (*e.g.*, Guillian-Barré syndrome, peripheral neuropathy, or exposure to environmental toxins or drugs).
In addition, the muscle atrophy can be a result of myopathy, such as myotonia; a congential myopathy, including nemalene myopathy, multi/minicore myopathy and myotubular (centronuclear) myopathy; mitochondrial myopathy; familial periodic paralysis; inflammatory myopathy; metabolic myopathy, such as caused by a glycogen or lipid storage disease; dermatomyositisis; polymyositis; inclusion body myositis; myositis ossificans; rhabdomyolysis and myoglobinurias. Furthermore, the term muscle atrophy also relates to obesity-associated sarcopenia, sarcopenia, or diabetes-associated muscle atrophy.
The myopathy may be caused by a muscular dystrophy syndrome, such as Duchenne, Becker, myotonic, fascioscapulohumeral, Emery-Dreifuss, oculopharyngeal, scapulohumeral, limb girdle, Fukuyama, a congenital muscular dystrophy, or hereditary distal myopathy. The musculoskeletal disease can also be osteoporosis, a bone fracture, short stature, or dwarfism.

In addition, the muscle atrophy can be a result of an adult motor neuron disease, infantile spinal muscular atrophy, amyotrophic lateral sclerosis, juvenile spinal muscular atrophy, autoimmune motor neuropathy with multifocal conductor block, paralysis due to stroke or spinal cord injury, skeletal immobilization due to trauma, prolonged bed rest, voluntary inactivity, involuntary inactivity, metabolic stress or nutritional insufficiency, cancer, AIDS, fasting, a thyroid gland disorder, benign congenital hypotonia, central core disease, burn injury, chronic obstructive pulmonary disease, liver diseases (examples such as fibrosis, cirrhosis), sepsis, renal failure, congestive heart failure, ageing, space travel or time spent in a zero gravity environment.
Examples of age-related conditions that may be treated include, sarcopenia, skin atrophy or muscle wasting.
The treatment might lead to the total absence of a metabolic disorder. In this context, the term "total absence" refers to a physiological conditions or status of a person which, on the basis of measurement of relevant physiological/metabolic parameter, would be considered by a skilled person as being healthy (all parameters are within the normal range). The skilled person is well aware of the relevant physiological/metabolic parameter and how to determine them. Said parameters will be selected by a skilled person (e.g. a physician) on the basis of the investigated age-related condition or metabolic disorder. The treatment results may also be partial, such that the peculiarity of the metabolic disorder in a subject is significantly less pronounced than had the subject not received a composition of the present invention. Partial treatment results may be a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. In this context, the term "significantly less pronounced metabolic disorder" refers to a physiological conditions or status of a person which, on the basis of measurement of relevant physiological/metabolic parameter, would after the treatment with the inventive compositions, not be considered by a skilled person as being completely healthy (parameters might be still outside the normal range), but where a significant improvement (which could be an increase or decrease of a certain parameter) of the relevant physiological/metabolic parameter has been observed. A significant improvement or decrease can be identified for example by comparison of the treatment results of individual patients compared to individuals of a control or placebo group.

[0051] Various aspects of the invention are described in further detail in the following subsections. Standard assays to evaluate the binding ability of the antibodies toward ActRIIB of various species are known in the art, including for example, ELISAs, western blots and RIAs. Suitable assays are described in detail in the Examples. The binding affinity of the antibodies also can be assessed by standard assays known in the art, such as by Biacore analysis or Solution Equilibrium Titration. Surface plasmon resonance based techniques such as Biacore can determine the binding kinetics which allows the calculation of the binding affinity. Assays to evaluate the effects of the antibodies on functional properties of ActRIIB (*e.g.* receptor binding, preventing or inducing human B cell proliferation or IgG production) are described in further detail in the Examples.
Accordingly, an antibody that "inhibits" one or more of these ActRIIB functional properties (*e.g.* biochemical, immunochemical, cellular, physiological or other biological activities, or the like) as

determined according to methodologies known to the art and described herein, will be understood to relate to a statistically significant decrease in the particular activity relative to that seen in the absence of the antibody (*e.g.* or when a control antibody of irrelevant specificity is present). An antibody that inhibits ActRIIB activity effects such a statistically significant decrease by at least 10% of the measured parameter, by at least 50%, 80% or 90%, and in certain embodiments an antibody of the invention may inhibit greater than 95%, 98% or 99% of ActRIIB functional activity.

The ability or extent to which an antibody or other binding agent is able to interfere with the binding of another antibody or binding molecule to ActRIIB, and therefore whether it can be said to cross-block according to the disclosure, can be determined using standard competition binding assays. One suitable assay involves the use of the Biacore technology (*e.g.* by using a BIAcore instrument (Biacore, Uppsala, Sweden)), which can measure the extent of interactions using surface plasmon resonance technology. Another assay for measuring cross-blocking uses an ELISA-based approach. A further assay uses FACS analysis, wherein competition of various antibodies for binding to ActRIIB expressing cells is tested (such as described in the Examples).

According to the disclosure, a cross-blocking antibody or other binding agent according to the disclosure binds to ActRIIB in the described BIAcore cross-blocking assay such that the recorded binding of the combination (mixture) of the antibodies or binding agents is between 80% and 0.1% (*e.g.* 80% to 4%) of the maximum theoretical binding, specifically between 75% and 0.1% (*e.g.* 75% to 4%) of the maximum theoretical binding, and more specifically between 70% and 0.1% (*e.g.* 70% to 4%), and more specifically between 65% and 0.1% (*e.g.* 65% to 4%) of maximum theoretical binding (as defined above) of the two antibodies or binding agents in combination.

An antibody is defined as cross-blocking an anti-ActRIIB antibody of the disclosure in an ELISA assay, if the test antibody is able to cause a reduction of anti-ActRIIB antibody binding to ActRIIB of between 60% and 100%, specifically between 70% and 100%, and more specifically between 80% and 100%, when compared to the positive control wells (*i.e.* the same anti-ActRIIB antibody and ActRIIB, but no "test" cross-blocking antibody). Examples of cross blocking antibodies as cited herein are MOR08159 and MOR08213. Thus, the invention provides compositions comprising antibodies that cross block MOR08159 or MOR08213 for binding to ActRIIB.

Recombinant antibodies

[0052] Antibodies comprised in the inventive compositions include the human recombinant antibodies, isolated and structurally characterized, as described in the Examples. The V_H amino acid sequences of antibodies comprised in the inventive compositions are shown in SEQ ID NOs: 99-112. The V_L amino acid sequences of antibodies comprised in the inventive compositions are shown in SEQ ID NOs: 85-98 respectively. Examples of preferred full length heavy chain amino acid sequences of antibodies comprised in the inventive compositions are

shown in SEQ ID NOs: 146-150 and 156-160. Examples of preferred full length light chain amino acid sequences of antibodies comprised in the inventive compositions are shown in SEQ ID NOs: 141-145 and 151-155 respectively. Other antibodies comprised in the inventive compositions include amino acids that have been mutated by amino acid deletion, insertion or substitution, yet have at least 60, 70, 80, 90, 95, 97 or 99 percent identity in the CDR regions with the CDR regions depicted in the sequences described above. In some embodiments, it includes mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated by amino acid deletion, insertion or substitution in the CDR regions when compared with the CDR regions depicted in the sequence described above.

[0053] Further, variable heavy chain parental nucleotide sequences are shown in SEQ ID NOs: 127-140. Variable light chain parental nucleotide sequences are shown in SEQ ID NOs: 113-126. Full length light chain nucleotide sequences optimized for expression in a mammalian cell are shown in SEQ ID NOs: 161-165 and 171-175. Full length heavy chain nucleotide sequences optimized for expression in a mammalian cell are shown in SEQ ID NOs: 166-170 and 176-180. Other antibodies comprised in the inventive compositions include amino acids or are encoded by nucleic acids that have been mutated, yet have at least 60 or more (*i.e.* 80, 90, 95, 97, 99 or more) percent identity to the sequences described above. In some embodiments, it includes mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated by amino acid deletion, insertion or substitution in the variable regions when compared with the variable regions depicted in the sequence described above.

[0054] Since each of these antibodies binds the same epitope and are progenies from the same parental antibody, the V_H, V_L, full length light chain, and full length heavy chain sequences (nucleotide sequences and amino acid sequences) can be "mixed and matched" to create other anti-ActRIIB binding molecules of the invention. ActRIIB binding of such "mixed and matched" antibodies can be tested using the binding assays described above and in the Examples (*e.g.* ELISAs). When these chains are mixed and matched, a V_H sequence from a particular V_H/V_L pairing should be replaced with a structurally similar V_H sequence. Likewise a full length heavy chain sequence from a particular full length heavy chain / full length light chain pairing should be replaced with a structurally similar full length heavy chain sequence. Likewise, a V_L sequence from a particular V_H/V_L pairing should be replaced with a structurally similar V_L sequence. Likewise a full length light chain sequence from a particular full length heavy chain / full length light chain pairing should be replaced with a structurally similar full length light chain sequence. Accordingly, in one aspect, the invention provides compositions comprising a recombinant anti-ActRIIB antibody or antigen binding region thereof having: a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 99-112; and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 85-98.

[0055] In another aspect, the invention provides compositions comprising:

1. (i) an isolated recombinant anti-ActRIIB antibody having: a full length heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID

NOs:99-112; and a full length light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:85-98, or

2. (ii) a functional protein comprising an antigen binding portion thereof.

[0056] In another aspect, the invention provides compositions comprising:

1. (i) an isolated recombinant anti-ActRIIB antibody having a full length heavy chain encoded by a nucleotide sequence that has been optimized for expression in the cell of a mammalian selected from the group consisting of SEQ ID NOs:127-140, and a full length light chain encoded by a nucleotide sequence that has been optimized for expression in the cell of a mammalian selected from the group consisting of SEQ ID NOs:113-126, or
2. (ii) a functional protein comprising an antigen binding portion thereof.

[0057] Examples of amino acid sequences of the V_H CDR1s of the antibodies comprised in the inventive compositions are shown in SEQ ID NOs: 1-14. The amino acid sequences of the V_H CDR2s of the antibodies are shown in SEQ ID NOs: 15-28. The amino acid sequences of the V_H CDR3s of the antibodies are shown in SEQ ID NOs: 29-42. The amino acid sequences of the V_L CDR1s of the antibodies are shown in SEQ ID NOs: 43-56. The amino acid sequences of the V_L CDR2s of the antibodies are shown in SEQ ID NOs: 57-70. The amino acid sequences of the V_L CDR3s of the antibodies are shown in SEQ ID NOs: 71-84. The CDR regions are delineated using the Kabat system (Kabat, E. A., et al., 1991 Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). An alternative method of determining CDR regions uses the method devised by Chothia (Chothia et al. 1989, Nature, 342:877-883). The Chothia definition is based on the location of the structural loop regions. However, due to changes in the numbering system used by Chothia (see e.g. http://www.biochem.ucl.ac.uk/~martin/abs/GeneralInfo.html and http://www.bioinf.org.uk/abs/), this system is now less commonly used. Other systems for defining CDRs exist and are also mentioned in these two websites.

[0058] Given that each of these antibodies can bind to ActRIIB and that antigen-binding specificity is provided primarily by the CDR1, 2 and 3 regions, the V_H CDR1, 2 and 3 sequences and V_L CDR1, 2 and 3 sequences can be "mixed and matched" (*i.e.* CDRs from different antibodies can be mixed and matched, each antibody containing a V_H CDR1, 2 and 3 and a V_L CDR1, 2 and 3 create other anti-ActRIIB binding molecules of the invention. ActRIIB binding of such "mixed and matched" antibodies can be tested using the binding assays described above and in the Examples (*e.g.* ELISAs). When V_H CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular V_H sequence should be

replaced with a structurally similar CDR sequence(s). Likewise, when V_L CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular V_L sequence should be replaced with a structurally similar CDR sequence(s). It will be readily apparent to the ordinarily skilled artisan that novel V_H and V_L sequences can be created by substituting one or more V_H and/or V_L CDR region sequences with structurally similar sequences from the CDR sequences shown herein for monoclonal antibodies .

[0059] Anti-ActRIIB antibody comprised in the inventive compositions, or antigen binding region thereof has: a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14; a heavy chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15-28; a heavy chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 29-42; a light chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 43-56; a light chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 57-70; and a light chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 71-84.

[0060] In one embodiment, the antibody comprised in the inventive composition comprises: a heavy chain variable region CDR1 of SEQ ID NO: 1; a heavy chain variable region CDR2 of SEQ ID NO: 15; a heavy chain variable region CDR3 of SEQ ID NO: 29; a light chain variable region CDR1 of SEQ ID NO: 43; a light chain variable region CDR2 of SEQ ID NO: 57; and a light chain variable region CDR3 of SEQ ID NO: 71.

[0061] In one embodiment, the antibody comprised in the inventive composition comprises: a heavy chain variable region CDR1 of SEQ ID NO: 2 a heavy chain variable region CDR2 of SEQ ID NO: 16; a heavy chain variable region CDR3 of SEQ ID NO: 30; a light chain variable region CDR1 of SEQ ID NO: 44; a light chain variable region CDR2 of SEQ ID NO: 58; and a light chain variable region CDR3 of SEQ ID NO: 72.

[0062] In one embodiment, the antibody comprised in the inventive composition comprises: a heavy chain variable region CDR1 of SEQ ID NO: 3; a heavy chain variable region CDR2 of SEQ ID NO: 17; a heavy chain variable region CDR3 of SEQ ID NO: 31; a light chain variable region CDR1 of SEQ ID NO: 45; a light chain variable region CDR2 of SEQ ID NO: 59; and a light chain variable region CDR3 of SEQ ID NO: 73.

[0063] In one embodiment, the antibody comprised in the inventive composition comprises: a heavy chain variable region CDR1 of SEQ ID NO: 4; a heavy chain variable region CDR2 of SEQ ID NO: 18; a heavy chain variable region CDR3 of SEQ ID NO: 32; a light chain variable region CDR1 of SEQ ID NO: 46; a light chain variable region CDR2 of SEQ ID NO: 60; and a light chain variable region CDR3 of SEQ ID NO: 74.

[0064] In one embodiment, the antibody comprised in the inventive composition comprises: a

heavy chain variable region CDR1 of SEQ ID NO: 5; a heavy chain variable region CDR2 of SEQ ID NO: 19; a heavy chain variable region CDR3 of SEQ ID NO: 33; a light chain variable region CDR1 of SEQ ID NO: 47; a light chain variable region CDR2 of SEQ ID NO: 61; and a light chain variable region CDR3 of SEQ ID NO: 75.

[0065] In one embodiment, the antibody comprised in the inventive composition comprises: a heavy chain variable region CDR1 of SEQ ID NO: 6; a heavy chain variable region CDR2 of SEQ ID NO: 20; a heavy chain variable region CDR3 of SEQ ID NO: 34; a light chain variable region CDR1 of SEQ ID NO: 48; a light chain variable region CDR2 of SEQ ID NO: 62; and a light chain variable region CDR3 of SEQ ID NO: 76.

[0066] In one embodiment, the antibody comprised in the inventive composition comprises: a heavy chain variable region CDR1 of SEQ ID NO: 7; a heavy chain variable region CDR2 of SEQ ID NO: 21; a heavy chain variable region CDR3 of SEQ ID NO: 35; a light chain variable region CDR1 of SEQ ID NO: 49; a light chain variable region CDR2 of SEQ ID NO: 63; and a light chain variable region CDR3 of SEQ ID NO: 77.

[0067] In one embodiment, the antibody comprised in the inventive composition comprises: a heavy chain variable region CDR1 of SEQ ID NO: 8; a heavy chain variable region CDR2 of SEQ ID NO: 22; a heavy chain variable region CDR3 of SEQ ID NO: 36; a light chain variable region CDR1 of SEQ ID NO: 50 a light chain variable region CDR2 of SEQ ID NO: 64; and a light chain variable region CDR3 of SEQ ID NO: 78.

[0068] In one embodiment, the antibody comprised in the inventive composition comprises: a heavy chain variable region CDR1 of SEQ ID NO: 9; a heavy chain variable region CDR2 of SEQ ID NO: 23; a heavy chain variable region CDR3 of SEQ ID NO: 37; a light chain variable region CDR1 of SEQ ID NO: 51; a light chain variable region CDR2 of SEQ ID NO: 65; and a light chain variable region CDR3 of SEQ ID NO: 79.

[0069] In one embodiment, the antibody comprised in the inventive composition comprises: a heavy chain variable region CDR1 of SEQ ID NO: 10; a heavy chain variable region CDR2 of SEQ ID NO: 24; a heavy chain variable region CDR3 of SEQ ID NO: 38; a light chain variable region CDR1 of SEQ ID NO: 52; a light chain variable region CDR2 of SEQ ID NO: 66; and a light chain variable region CDR3 of SEQ ID NO: 80.

[0070] In one embodiment, the antibody comprised in the inventive composition comprises: a heavy chain variable region CDR1 of SEQ ID NO: 11; a heavy chain variable region CDR2 of SEQ ID NO: 25; a heavy chain variable region CDR3 of SEQ ID NO: 39; a light chain variable region CDR1 of SEQ ID NO: 53; a light chain variable region CDR2 of SEQ ID NO: 67; and a light chain variable region CDR3 of SEQ ID NO: 81.

[0071] In one embodiment, the antibody comprised in the inventive composition comprises: a heavy chain variable region CDR1 of SEQ ID NO: 12; a heavy chain variable region CDR2 of SEQ ID NO: 26; a heavy chain variable region CDR3 of SEQ ID NO: 40; a light chain variable

region CDR1 of SEQ ID NO: 54; a light chain variable region CDR2 of SEQ ID NO: 68; and a light chain variable region CDR3 of SEQ ID NO: 82.

[0072] In one embodiment, the antibody comprised in the inventive composition comprises: a heavy chain variable region CDR1 of SEQ ID NO: 13; a heavy chain variable region CDR2 of SEQ ID NO: 27; a heavy chain variable region CDR3 of SEQ ID NO: 41; a light chain variable region CDR1 of SEQ ID NO: 55; a light chain variable region CDR2 of SEQ ID NO: 69; and a light chain variable region CDR3 of SEQ ID NO: 83.

[0073] In one embodiment, the antibody comprised in the inventive composition comprises: a heavy chain variable region CDR1 of SEQ ID NO: 14; a heavy chain variable region CDR2 of SEQ ID NO: 28; a heavy chain variable region CDR3 of SEQ ID NO: 42; a light chain variable region CDR1 of SEQ ID NO: 56; a light chain variable region CDR2 of SEQ ID NO: 70; and a light chain variable region CDR3 of SEQ ID NO: 84.

[0074] In one embodiment, the invention provides a composition comprising an antibody comprising: (a) the variable heavy chain sequence of SEQ ID NO: 85 and variable light chain sequence of SEQ ID NO: 99; (b) the variable heavy chain sequence of SEQ ID NO: 86 and variable light chain sequence of SEQ ID NO: 100; (c) the variable heavy chain sequence of SEQ ID NO: 87 and variable light chain sequence of SEQ ID NO: 101; (d) the variable heavy chain sequence of SEQ ID NO: 88 and variable light chain sequence of SEQ ID NO: 102; (e) the variable heavy chain sequence of SEQ ID NO: 89 and variable light chain sequence of SEQ ID NO: 103; (f) the variable heavy chain sequence of SEQ ID NO: 90 and variable light chain sequence of SEQ ID NO: 104; (g) the variable heavy chain sequence of SEQ ID NO: 91 and variable light chain sequence of SEQ ID NO: 105; (h) the variable heavy chain sequence of SEQ ID NO: 92 and variable light chain sequence of SEQ ID NO: 106; (i) the variable heavy chain sequence of SEQ ID NO: 93 and variable light chain sequence of SEQ ID NO: 107; (j) the variable heavy chain sequence of SEQ ID NO: 94 and variable light chain sequence of SEQ ID NO: 108; (k) the variable heavy chain sequence of SEQ ID NO: 95 and variable light chain sequence of SEQ ID NO: 109; (l) the variable heavy chain sequence of SEQ ID NO: 96 and variable light chain sequence of SEQ ID NO: 110; (m) the variable heavy chain sequence of SEQ ID NO: 97 and variable light chain sequence of SEQ ID NO: 111; or (n) the variable heavy chain sequence of SEQ ID NO: 98 and variable light chain sequence of SEQ ID NO: 112.

[0075] In one embodiment, the invention provides a composition comprising an antibody comprising: (a) the heavy chain sequence of SEQ ID NO: 146 and light chain sequence of SEQ ID NO: 141; (b) the heavy chain sequence of SEQ ID NO: 147 and light chain sequence of SEQ ID NO: 142; (c) the heavy chain sequence of SEQ ID NO: 148 and light chain sequence of SEQ ID NO: 143; (d) the heavy chain sequence of SEQ ID NO: 149 and light chain sequence of SEQ ID NO: 144; (e) the heavy chain sequence of SEQ ID NO: 150 and light chain sequence of SEQ ID NO: 145; (f) the heavy chain sequence of SEQ ID NO: 156 and light chain sequence of SEQ ID NO: 151; (g) the heavy chain sequence of SEQ ID NO: 157 and light chain sequence of SEQ ID NO: 152; (h) the heavy chain sequence of SEQ ID NO: 158 and light chain sequence of SEQ ID NO: 153; (i) the heavy chain sequence of SEQ ID NO: 159 and

light chain sequence of SEQ ID NO: 154; or (j) the heavy chain sequence of SEQ ID NO: 160 and light chain sequence of SEQ ID NO: 155.

[0076] As used herein, a human antibody comprises heavy or light chain variable regions or full length heavy or light chains that are "the product of" or "derived from" a particular germline sequence if the variable regions or full length chains of the antibody are obtained from a system that uses human germline immunoglobulin genes. Such systems include immunizing a transgenic mouse carrying human immunoglobulin genes with the antigen of interest or screening a human immunoglobulin gene library displayed on phage with the antigen of interest. A human antibody that is "the product of" or "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequences of human germline immunoglobulins and selecting the human germline immunoglobulin sequence that is closest in sequence (*i.e.* greatest % identity) to the sequence of the human antibody. A human antibody that is "the product of" or "derived from" a particular human germline immunoglobulin sequence may contain amino acid differences as compared to the germline sequence, due to, for example, naturally occurring somatic mutations or intentional introduction of site-directed mutation. However, a selected human antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the human antibody as being human when compared to the germline immunoglobulin amino acid sequences of other species (*e.g.* murine germline sequences). In certain cases, a human antibody may be at least 80%, 90%, or at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a human antibody derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the human antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

[0077] In one embodiment the antibody comprised in the compositions of the invention is that encoded by pBW522 or pBW524 (deposited at DSMZ, Inhoffenstr. 7B, D-38124 Braunschweig, Germany on 18 August 2009 under deposit numbers DSM22873 and DSM22874, respectively).

Homologous antibodies

[0078] In yet another embodiment, an antibody comprised in the inventive composition has full length heavy and light chain amino acid sequences; full length heavy and light chain nucleotide sequences, variable region heavy and light chain nucleotide sequences, or variable region heavy and light chain amino acid sequences that are homologous to the amino acid and nucleotide sequences of the antibodies described herein, and wherein the antibodies retain the desired functional properties of the anti-ActRIIB antibodies of the invention.

For example, the invention provides a composition comprising an isolated recombinant anti-ActRIIB antibody (or a functional protein comprising an antigen binding portion thereof) comprising a heavy chain variable region and a light chain variable region, wherein: the heavy chain variable region comprises an amino acid sequence that is at least 80%, or at least 90% (preferably at least 95, 97 or 99%) identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 99-112; the light chain variable region comprises an amino acid sequence that is at least 80%, or at least 90% (preferably at least 95, 97 or 99%) identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 85-98; alternatively the compositions comprises a recombinant anti-ActRIIB antibody (or a functional protein comprising an antigen binding portion thereof) comprising a heavy chain variable region and a light chain variable region, wherein: the heavy chain variable region comprises no more than 5 amino acid, or no more than 4 amino acid, or no more than 3 amino acid, or no more than 2 or no more than 1 amino acid change compared to the amino acid sequence selected from the group consisting of SEQ ID NOs: 99-112; the light chain variable region comprises no more than 5 amino acid, or no more than 4 amino acid, or no more than 3 amino acid, or no more than 2 or no more than 1 amino acid change compared to the amino acid sequence selected from the group consisting of SEQ ID NOs: 85-98 and the antibody exhibits at least one of the following functional properties: (i) it inhibits myostatin binding *in vitro* or *in vivo,* (ii) decreases inhibition of muscle differentiation through the Smad-dependent pathway and/or (iii) does not induce hematological changes, in particular no changes in RBC. In this context, the term "change" refers to insertions, deletions and/or substitutions.

In a further example, the invention provides a composition comprising an isolated recombinant anti-ActRIIB antibody, (or a functional protein comprising an antigen binding portion thereof) comprising a full length heavy chain and a full length light chain, wherein: the full length heavy chain comprises an amino acid sequence that is at least 80%, or at least 90% (preferably at least 95, 97 or 99%) identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 146-150 and 156-160; the full length light chain comprises an amino acid sequence that is at least 80%, or at least 90% (preferably at least 95, 97 or 99%) identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 141-145 and 151-155; alternatively the compositions comprises a recombinant anti-ActRIIB antibody (or a functional protein comprising an antigen binding portion thereof) comprising a heavy chain variable region and a light chain variable region, wherein: the heavy chain variable region comprises no more than 5 amino acid, or no more than 4 amino acid, or no more than 3 amino acid, or no more than 2 or no more than 1 amino acid change compared to the amino acid sequence selected from the group consisting of SEQ ID NOs: 146-150 and 156-160; the light chain variable region comprises no more than 5 amino acid, or no more than 4 amino acid, or no more than 3 amino acid, or no more than 2 or no more than 1 amino acid change compared to the amino acid sequence selected from the group consisting of SEQ ID NOs: 141-145 and 151-155 and the antibody exhibits at least one of the following functional properties: (i) it inhibits myostatin binding *in vitro* or *in vivo,* (ii) decreases inhibition of muscle differentiation through the Smad-dependent pathway and/or (iii) does not induce hematological changes, in particular no changes in RBC. Preferably such an antibody binds to the ligand binding domain of ActRIIB. In this context, the term "change" refers to insertions, deletions and/or substitutions.

[0079] In another example, the invention provides a composition comprising an isolated recombinant anti-ActRIIB antibody (or a functional protein comprising an antigen binding portion thereof), comprising a full length heavy chain and a full length light chain, wherein: the full length heavy chain is encoded by a nucleotide sequence that is at least 80%, or at least 90% (preferably at least 95, 97 or 99%) identical to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 166-170 and 176-180; the full length light chain is encoded by a nucleotide sequence that is at least 80%, or at least 90% (preferably at least 95, 97 or 99%) identical to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 161-165 and 171-175; alternatively the compositions comprises a recombinant anti-ActRIIB antibody (or a functional protein comprising an antigen binding portion thereof) comprising a heavy chain variable region and a light chain variable region, wherein: the heavy chain variable region comprises no more than 5 amino acid, or no more than 4 amino acid, or no more than 3 amino acid, or no more than 2 or no more than 1 amino acid change compared to the amino acid sequence selected from the group consisting of SEQ ID NOs: 166-170 and 176-180; the light chain variable region comprises no more than 5 amino acid, or no more than 4 amino acid, or no more than 3 amino acid, or no more than 2 or no more than 1 amino acid change compared to the amino acid sequence selected from the group consisting of SEQ ID NOs: 161-165 and 171-175 and the antibody exhibits at least one of the following functional properties: (i) it inhibits myostatin binding *in vitro* or *in vivo,* (ii) decreases inhibition of muscle differentiation through the Smad-dependent pathway and/or (iii) does not induce hematological changes, in particular no changes in RBC. Preferably such an antibody binds to the ligand binding domain of ActRIIB. In this context, the term "change" refers to insertions, deletions and/or substitutions.

[0080] In various embodiments, the antibody comprised in the inventive composition may exhibit one or more, two or more, or three of the functional properties discussed above. The antibody can be, for example, a human antibody, a humanized antibody or a chimeric antibody. Preferably the antibody is a fully human IgG1 antibody.

In other embodiments, the V_H and/or V_L amino acid sequences may be 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to the sequences set forth above. In other embodiments, the V_H and/or V_L amino acid sequences may be identical except an amino acid substitution in no more than 1, 2, 3, 4 or 5 amino acid position. An antibody having V_H and V_L regions having high (*i.e.* 80% or greater) identity to the V_H and V_L regions of SEQ ID NOs 99-112 and SEQ ID NOs: 85-98 respectively, can be obtained by mutagenesis (*e.g.* site-directed or PCR-mediated mutagenesis) of nucleic acid molecules SEQ ID NOs: 127-140 and 113-126 respectively, followed by testing of the encoded altered antibody for retained function (*i.e.* the functions set forth above) using the functional assays described herein.

In other embodiments, the full length heavy chain and/or full length light chain amino acid sequences may be 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to the sequences set forth above or may be identical except an amino acid change in no more than 1, 2, 3, 4 or 5 amino acid position. An antibody having a full length heavy chain and full length light chain having high (*i.e.* 80% or greater) identity to the full length heavy chains of any of SEQ ID NOs: 146-150 and 156-160 and full length light chains of any of SEQ ID NOs: 141-145 and 151-155

respectively, can be obtained by mutagenesis (*e.g.* site-directed or PCR-mediated mutagenesis) of nucleic acid molecules SEQ ID NOs: 166-170 and 176-180 and SEQ ID NOs: 161-165 and 171-175 respectively, followed by testing of the encoded altered antibody for retained function (*i.e.* the functions set forth above) using the functional assays described herein.

In other embodiments, the full length heavy chain and/or full length light chain nucleotide sequences may be 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to the sequences set forth above.

In other embodiments, the variable regions of heavy chain and/or light chain nucleotide sequences may be 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to the sequences set forth above or may be identical except an amino acid change in no more than 1, 2, 3, 4 or 5 amino acid position.

As used herein, the percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.* % identity = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described below.

The percent identity between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4:11-17, 1988) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol, Biol. 48:444-453, 1970) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

Antibodies with conservative modifications

[0081] In certain embodiments, an antibody comprised in the inventive composition has a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences, wherein one or more of these CDR sequences have specified amino acid sequences based on the antibodies described herein or variant sequences thereof comprising 1, 2, 3, 4 or 5 amino acid changes or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the anti-ActRIIB antibodies of the invention. Accordingly, the invention provides compositions comprising an isolated recombinant anti-ActRIIB antibody, or a functional protein comprising an antigen binding portion thereof, consisting of a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences, wherein: the heavy chain variable region CDR1 amino acid sequences are selected from the group consisting of SEQ ID NOs: 1-14 or variant sequences thereof comprising 1, 2, 3, 4 or 5 amino acid changes, and conservative

modifications thereof; the heavy chain variable region CDR2 amino acid sequences are selected from the group consisting of SEQ ID NOs: 15-28 or variant sequences thereof comprising 1, 2, 3, 4 or 5 amino acid changes, and conservative modifications thereof; the heavy chain variable region CDR3 amino acid sequences are selected from the group consisting of SEQ ID NOs: 29-42 or variant sequences thereof comprising 1, 2, 3, 4 or 5 amino acid changes, and conservative modifications thereof; the light chain variable regions CDR1 amino acid sequences are selected from the group consisting of SEQ ID NOs: 43-56 or variant sequences thereof comprising 1, 2, 3, 4 or 5 amino acid changes, and conservative modifications thereof; the light chain variable regions CDR2 amino acid sequences are selected from the group consisting of SEQ ID NOs: 57-70 or variant sequences thereof comprising 1, 2, 3, 4 or 5 amino acid changes, and conservative modifications thereof; the light chain variable regions of CDR3 amino acid sequences are selected from the group consisting of SEQ ID NOs: 71-84 or variant sequences thereof comprising 1, 2, 3, 4 or 5 amino acid changes, and conservative modifications thereof. Preferably the antibody exhibits at least one of the following functional properties: (i) it inhibits myostatin binding *in vitro* or *in vivo,* (ii) decreases inhibition of muscle differentiation through the Smad-dependent pathway and/or (iii) does not induce hematological changes, in particular no changes in RBC.

[0082] In various embodiments, the antibody may exhibit one or both of the functional properties listed above. Such antibodies can be, for example, human antibodies, humanized antibodies or chimeric antibodies.

[0083] In other embodiments, an antibody comprised in the inventive composition optimized for expression in a mammalian cell has a full length heavy chain sequence and a full length light chain sequence, wherein one or more of these sequences have specified amino acid sequences based on the antibodies described herein or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the anti-ActRIIB antibodies of the invention. Accordingly, the invention provides compositions comprising an isolated monoclonal anti-ActRIIB antibody optimized for expression in a mammalian cell consisting of a full length heavy chain and a full length light chain wherein: the full length heavy chain has amino acid sequences selected from the group of SEQ ID NOs: 146-150 and 156-160 or variant sequences thereof comprising 1, 2, 3, 4 or 5 amino acid changes, and conservative modifications thereof; and the full length light chain has amino acid sequences selected from the group of SEQ ID NOs: 141-145 and 151-155 or variant sequences thereof comprising 1, 2, 3, 4 or 5 amino acid changes, and conservative modifications thereof; and the antibody exhibits at least one of the following functional properties: (i) it inhibits myostatin binding *in vitro* or *in vivo,* (ii) decreases inhibition of muscle differentiation through the Smad-dependent pathway and/or (iii) does not induce hematological changes, in particular no changes in RBC.

[0084] In various embodiments, the antibody may exhibit one or both of the functional properties listed above. Such antibodies can be, for example, human antibodies, humanized antibodies or chimeric antibodies.

[0085] As used herein, the term "conservative sequence modifications" is intended to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis.

[0086] Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.* lysine, arginine, histidine), acidic side chains (*e.g.* aspartic acid, glutamic acid), uncharged polar side chains (*e.g.* glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.* alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.* threonine, valine, isoleucine) and aromatic side chains (*e.g.* tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within the CDR regions of an antibody of the invention can be replaced with other amino acid residues from the same side chain family, and the altered antibody can be tested for retained function using the functional assays described herein.

[0087] Antibodies that bind to the same epitope as anti-ActRIIB antibodies comprised in the inventive composition

[0088] In another embodiment, the invention provides compositions comprising antibodies that bind to the same epitope as the various specific anti-ActRIIB antibodies described herein. All the antibodies described in the examples that are capable of blocking myostatin binding to ActRIIB bind to one of the epitopes in ActRIIB with high affinity, said epitope being comprised between amino acids 19-134 of SEQ ID NO:181.

[0089] Additional antibodies can therefore be identified based on their ability to cross-compete (*e.g.* to competitively inhibit the binding of, in a statistically significant manner) with other antibodies of the disclosure in standard ActRIIB binding assays. The ability of a test antibody to inhibit the binding of antibodies comprised in the inventive compositions to human ActRIIB demonstrates that the test antibody can compete with said antibody for binding to human ActRIIB; such an antibody may, according to non-limiting theory, bind to the same or a related (*e.g.* a structurally similar or spatially proximal) epitope on human ActRIIB as the antibody with which it competes. In a certain embodiment, the antibody that binds to the same epitope on human ActRIIB as the antibodies comprised in the inventive compositions is a human recombinant antibody. Such human recombinant antibodies can be prepared and isolated as described in the examples.

[0090] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by and/or that competes for binding with an antibody having the variable heavy chain sequence recited in SEQ ID NO: 85, and the variable light chain sequence recited

in SEQ ID NO: 99.

[0091] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by an antibody having the variable heavy chain sequence recited in SEQ ID NO: 86, and the variable light chain sequence recited in SEQ ID NO: 100.

[0092] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by an antibody having the variable heavy chain sequence recited in SEQ ID NO: 87, and the variable light chain sequence recited in SEQ ID NO: 101.

[0093] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by an antibody having the variable heavy chain sequence recited in SEQ ID NO: 88, and the variable light chain sequence recited in SEQ ID NO: 102.

[0094] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by an antibody having the variable heavy chain sequence recited in SEQ ID NO: 89, and the variable light chain sequence recited in SEQ ID NO: 103.

[0095] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by an antibody having the variable heavy chain sequence recited in SEQ ID NO: 90, and the variable light chain sequence recited in SEQ ID NO: 104.

[0096] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by an antibody having the variable heavy chain sequence recited in SEQ ID NO: 91, and the variable light chain sequence recited in SEQ ID NO: 105.

[0097] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by an antibody having the variable heavy chain sequence recited in SEQ ID NO: 92, and the variable light chain sequence recited in SEQ ID NO: 106.

[0098] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by an antibody having the variable heavy chain sequence recited in SEQ ID NO: 93, and the variable light chain sequence recited in SEQ ID NO: 107.

[0099] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by an antibody having the variable heavy chain sequence recited in SEQ ID NO: 94, and the variable light chain sequence recited in SEQ ID NO: 108.

[0100] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by an antibody having the variable heavy chain sequence recited in SEQ ID NO: 95, and the variable light chain sequence recited in SEQ ID NO: 109.

[0101] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by an antibody having the variable heavy chain sequence recited in SEQ ID

NO: 96, and the variable light chain sequence recited in SEQ ID NO: 110.

[0102] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by an antibody having the variable heavy chain sequence recited in SEQ ID NO: 97, and the variable light chain sequence recited in SEQ ID NO: 111.

[0103] Thus, the invention provides a composition comprising an antibody that binds to an epitope recognised by an antibody having the variable heavy chain sequence recited in SEQ ID NO: 98, and the variable light chain sequence recited in SEQ ID NO: 112.

[0104] Following more detailed epitope mapping experiments, the binding regions of preferred antibodies of the inventive compositions have been more clearly defined.

[0105] Thus, the invention provides a composition comprising an antibody that binds to an epitope comprising amino acids 78-83 of SEQ ID NO: 181 (WLDDFN - SEQ ID NO:188).

[0106] The invention also provides a composition comprising an antibody that binds to an epitope comprising amino acids 76-84 of SEQ ID NO: 181 (GCWLDDFNC - SEQ ID NO:186).

[0107] The invention also provides a composition comprising an antibody that binds to an epitope comprising amino acids 75-85 of SEQ ID NO: 181 (KGCWLDDFNCY - SEQ ID NO:190). The invention also provides a composition comprising an antibody that binds to an epitope comprising amino acids 52-56 of SEQ ID NO: 181 (EQDKR - SEQ ID NO:189).

[0108] The invention also provides a composition comprising an antibody that binds to an epitope comprising amino acids 49-63 of SEQ ID NO: 181 (CEGEQDKRLHCYASW - SEQ ID NO:187).

[0109] The invention also provides a composition comprising an antibody that binds to an epitope comprising or consisting of amino acids 29-41 of SEQ ID NO: 181 (CIYYNANWELERT- SEQ ID NO:191).

[0110] The invention also provides a composition comprising an antibody that binds to an epitope comprising or consisting of amino acids 100-110 of SEQ ID NO: 181 (YFCCCEGNFCN - SEQ ID NO:192); or

[0111] The invention also provides a composition comprising antibodies that bind to epitopes consisting of these sequences or epitopes comprising combinations of these epitope regions.

[0112] Thus, the invention also provides a composition comprising an antibody that binds to an epitope comprising or consisting of amino acids 78-83 of SEQ ID NO: 181 (WLDDFN) and amino acids 52-56 of SEQ ID NO: 181 (EQDKR).

Engineered and modified antibodies

[0113] An antibody comprised in the inventive compositions further can be prepared using an antibody having one or more of the V_H and/or V_L sequences shown herein as starting material to engineer a modified antibody, which modified antibody may have altered properties from the starting antibody. An antibody can be engineered by modifying one or more residues within one or both variable regions (*i.e.* V_H and/or V_L), for example within one or more CDR regions and/or within one or more framework regions. Additionally or alternatively, an antibody can be engineered by modifying residues within the constant region(s), for example to alter the effector function(s) of the antibody.

[0114] One type of variable region engineering that can be performed is CDR grafting. Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, *e.g.* Riechmann, L. et al., 1998 Nature 332:323-327; Jones, P. et al., 1986 Nature 321:522-525; Queen, C. et al., 1989 Proc. Natl. Acad. Sci. U.S.A. 86:10029-10033; U.S. Patent No. 5,225,539 to Winter, and U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen et al.).

[0115] Accordingly, another embodiment of the invention pertains to compositions comprising a monoclonal anti- ActRIIB antibody, or a functional protein comprising an antigen binding portion thereof, comprising a heavy chain variable region comprising CDR1 sequences having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-14; CDR2 sequences having an amino acid sequence selected from the group consisting of SEQ ID NOs: 15-28; CDR3 sequences having an amino acid sequence selected from the group consisting of SEQ ID NOs: 29-42, respectively; and a light chain variable region having CDR1 sequences having an amino acid sequence selected from the group consisting of SEQ ID NOs: 43-56; CDR2 sequences having an amino acid sequence selected from the group consisting of SEQ ID NOs: 57-70; and CDR3 sequences consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 71-84, respectively. Thus, such antibodies contain the V_H and V_L CDR sequences of monoclonal antibodies, yet may contain different framework sequences from these antibodies.

[0116] Such framework sequences can be obtained from public DNA databases or published references that include germline antibody gene sequences. For example, germline DNA sequences for human heavy and light chain variable region genes can be found in the "VBase" human germline sequence database (available on the Internet at www.mrc-cpe.cam.ac.uk/vbase), as well as in Kabat, E. A., *et al.*, [*supra*]; Tomlinson, I. M., et al., 1992 J.

fol. Biol. 227:776-798; and Cox, J. P. L. et al., 1994 Eur. J Immunol. 24:827-836. An example of framework sequences for use in the antibodies of the invention are those that are structurally similar to the framework sequences used by selected antibodies of the invention, *e.g.* consensus sequences and/or framework sequences used by monoclonal antibodies of the invention. The V_H CDR1, 2 and 3 sequences, and the V_L CDR1, 2 and 3 sequences, can be grafted onto framework regions that have the identical sequence as that found in the germline immunoglobulin gene from which the framework sequence derive, or the CDR sequences can be grafted onto framework regions that contain one or more mutations as compared to the germline sequences. For example, it has been found that in certain instances it is beneficial to mutate residues within the framework regions to maintain or enhance the antigen binding ability of the antibody (see *e.g.* U.S. Patents. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen et al).

[0117] Another type of variable region modification is to mutate amino acid residues within the V_H and/or V_L CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (*e.g.* affinity) of the antibody of interest, known as "affinity maturation." Site-directed mutagenesis or PCR-mediated mutagenesis can be performed to introduce the mutation(s) and the effect on antibody binding, or other functional property of interest, can be evaluated in *in vitro* or *in vivo* assays as described herein and provided in the Examples. Conservative modifications (as discussed above) can be introduced. The mutations may be amino acid substitutions, additions or deletions. Moreover, typically no more than one, two, three, four or five residues within a CDR region are altered.

[0118] Accordingly, in another embodiment, the invention provides isolated anti-ActRIIB monoclonal antibodies, or a functional protein comprising an antigen binding portion thereof, consisting of a heavy chain variable region having: a V_H CDR1 region consisting of an amino acid sequence selected from the group having SEQ ID NOs: 1-14 or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 1-14; a V_H CDR2 region having an amino acid sequence selected from the group consisting of SEQ ID NOs: 15-28, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 15-28; a V_H CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NOs: 29-42, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 29-42; a V_L CDR1 region having an amino acid sequence selected from the group consisting of SEQ ID NOs: 43-56, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 43-56; a V_L CDR2 region having an amino acid sequence selected from the group consisting of SEQ ID NOs: 52-70, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 52-70; and a V_L CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NOs: 71-84, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 71-84.

[0119] Grafting antigen-binding domains into alternative frameworks or scaffolds

[0120] A wide variety of antibody/immunoglobulin frameworks or scaffolds can be employed so long as the resulting polypeptide includes at least one binding region which specifically binds to ActRIIB. Such frameworks or scaffolds include the 5 main idiotypes of human immunoglobulins, or fragments thereof (such as those disclosed elsewhere herein), and include immunoglobulins of other animal species, preferably having humanized aspects. Single heavy-chain antibodies such as those identified in camelids are of particular interest in this regard. Novel frameworks, scaffolds and fragments continue to be discovered and developed by those skilled in the art.

[0121] In one aspect, the compositions of the invention may comprise non-immunoglobulin based antibodies using non-immunoglobulin scaffolds onto which CDRs of the disclosed antibodies can be grafted. Known or future non-immunoglobulin frameworks and scaffolds may be employed, as long as they comprise a binding region specific for the target protein of SEQ ID NO: 181 (preferably, the ligand binding domain thereof as shown in SEQ ID NO: 182). Such compounds are known herein as "polypeptides comprising a target-specific binding region".

Framework or Fc engineering

[0122] Engineered antibodies comprised in the compositions of the invention include those in which modifications have been made to framework residues within V_H and/or V_L, *e.g.* to improve the properties of the antibody. Typically such framework modifications are made to decrease the immunogenicity of the antibody. For example, one approach is to "backmutate" one or more framework residues to the corresponding germline sequence. More specifically, an antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived. To return the framework region sequences to their germline configuration, the somatic mutations can be "backmutated" to the germline sequence by, for example, site-directed mutagenesis or PCR-mediated mutagenesis. Such "backmutated" antibodies can also be comprised in the compositions of the invention.

[0123] Another type of framework modification involves mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T-cell epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as "deimmunization" and is described in further detail in US2003/0153043.

[0124] In addition or alternative to modifications made within the framework or CDR regions, antibodies of the invention may be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, an antibody comprised in the compositions of the invention may be chemically

modified (*e.g.* one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody. Each of these embodiments is described in further detail below. The numbering of residues in the Fc region is that of the EU index of Kabat.

[0125] In one embodiment, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, *e.g.* increased or decreased. This approach is described further in US5,677,425. The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

[0126] In another embodiment, the Fc hinge region of an antibody is mutated to decrease the biological half-life of the antibody. More specifically, one or more amino acid mutations are introduced into the CH2-CH3 domain interface region of the Fc-hinge fragment such that the antibody has impaired Staphylococcyl protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in US 6,165,745. In another embodiment, the antibody is modified to increase its biological half-life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in US6,277,375. Alternatively, to increase the biological half life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in US5,869,046 and US6,121,022.

[0127] In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector functions of the antibody. For example, one or more amino acids can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in US5,624,821 and US5,648,260, both by Winter et al. In particular, residues 234 and 235 may be mutated. In particular, these mutations may be to alanine. Thus in one embodiment the antibody comprised in the compositions of the invention has a mutation in the Fc region at one or both of amino acids 234 and 235. In another embodiment, one or both of amino acids 234 and 235 may be substituted to alanine. Substitution of both amino acids 234 and 235 to alanine results in a reduced ADCC activity.

[0128] In another embodiment, one or more amino acids selected from amino acid residues of the described antibodies can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in US6,194,551.

[0129] In another embodiment, one or more amino acid residues of the described antibodies are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in WO94/29351.

[0130] In yet another embodiment, the Fc region of the described antibodies is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids. This approach is described further in WO00/42072. Moreover, the binding sites on human IgG1 for FcγRI, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields, R.L. et al., 2001 J. Biol. Chen. 276:6591-6604).

[0131] In still another embodiment, the glycosylation of an antibody comprised in the compositions of the invention is modified. For example, an aglycoslated antibody can be made (*i.e.* the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for the antigen. Such carbohydrate modifications can be accomplished by; for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such a glycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in U.S. Patent Nos. 5,714,350 and 6,350,861 by Co et al.

[0132] Additionally or alternatively, an antibody can be used that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express the disclosed recombinant antibodies to thereby produce an antibody with altered glycosylation. For example, EP 1,176,195 by Hang et al. describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation. Therefore, in one embodiment, the antibodies comprised in the compositions of the invention are produced by recombinant expression in a cell line which exhibit hypofucosylation pattern, for example, a mammalian cell line with deficient expression of the FUT8 gene encoding fucosyltransferase. WO03/035835 describes a variant CHO cell line, Lecl3 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, R.L. et al., 2002 J. Biol. Chem. 277:26733-26740). WO99/54342 describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (*e.g.* beta(1,4)-N acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana et al., 1999 Nat. Biotech. 17:176-180). Alternatively, the antibodies comprised in the compositions of the invention can be produced in a yeast or a filamentous fungi engineered for mammalian-like glycosylation pattern, and capable of producing antibodies lacking fucose as glycosylation pattern (see for example EP1297172B1).

[0133] Another modification of the antibodies herein that is contemplated by the invention is pegylation. An antibody can be pegylated to, for example, increase the biological (*e.g.* serum) half-life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. The pegylation can be carried out by an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain embodiments, the used antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the disclosed antibodies (see for example, EP0154316 and EP0401384).

[0134] Another modification of the antibodies that is contemplated by the invention is a conjugate or a protein fusion of at least the antigen-binding region of the antibody comprised in the composition of the invention to serum protein, such as human serum albumin or a fragment thereof to increase half-life of the resulting molecule (see, for example, EP0322094). Another possibility is a fusion of at least the antigen-binding region of the antibody comprised in the composition of the invention to proteins capable of binding to serum proteins, such as human serum albumin to increase half life of the resulting molecule (see, for example, EP0486525).

Methods of engineering altered antibodies

[0135] As discussed above, the anti-ActRIIB antibodies having CDR sequences, V_H and V_L sequences or full length heavy and light chain sequences shown herein can be used to create new anti-ActRIIB antibodies by modifying the CDR sequences full length heavy chain and/or light chain sequences, V_H and/or V_L sequences, or the constant region(s) attached thereto. Thus, in another aspect of the invention, the structural features of an anti-ActRIIB antibody comprised in the compositions of the invention are used to create structurally related anti-ActRIIB antibodies that retain at least one functional property of the antibodies comprised in the compositions of the invention, such as binding to human ActRIIB but also inhibit one or more functional properties of ActRIIB (for example, the inhibition of Smad activation).

[0136] For example, one or more CDR regions of the antibodies comprised in the compositions of the present invention, or mutations thereof, can be combined recombinantly with known framework regions and/or other CDRs to create additional, recombinantly-engineered, anti-ActRIIB antibodies comprised in the compositions of the invention, as discussed above. Other types of modifications include those described in the previous section. The starting material for the engineering method is one or more of the V_H and/or V_L sequences provided herein, or one or more CDR regions thereof. To create the engineered antibody, it is

not necessary to actually prepare (*i.e.* express as a protein) an antibody having one or more of the V_H and/or V_L sequences provided herein, or one or more CDR regions thereof. Rather, the information contained in the sequence(s) is used as the starting material to create a "second generation" sequence(s) derived from the original sequence(s) and then the "second generation" sequence(s) is prepared and expressed as a protein.

[0137] The altered antibody sequence can also be prepared by screening antibody libraries having fixed CDR3 sequences selected among the group consisting of SEQ ID NO: 29-42 and SEQ ID NO: 71-84 or minimal essential binding determinants as described in US2005/0255552 and diversity on CDR1 and CDR2 sequences. The screening can be performed according to any screening technology appropriate for screening antibodies from antibody libraries, such as phage display technology.

[0138] Standard molecular biology techniques can be used to prepare and express the altered antibody sequence. The antibody encoded by the altered antibody sequence(s) is one that retains one, some or all of the functional properties of the anti-ActRIIB antibodies described herein, which functional properties include, but are not limited to, specifically binding to human ActRIIB and inhibition of Smad activation.

[0139] The altered antibody may exhibit one or more, two or more, or three or more of the functional properties discussed above.

[0140] The functional properties of the altered antibodies can be assessed using standard assays available in the art and/or described herein, such as those set forth in the Examples (*e.g.* ELISAs).

[0141] Mutations can be introduced randomly or selectively along all or part of an anti-ActRIIB antibody coding sequence and the resulting modified anti-ActRIIB antibodies can be screened for binding activity and/or other functional properties as described herein. Mutational methods have been described in the art. For example, WO02/092780 describes methods for creating and screening antibody mutations using saturation mutagenesis, synthetic ligation assembly, or a combination thereof. Alternatively, WO03/074679 describes methods of using computational screening methods to optimize physiochemical properties of antibodies.

[0142] Nucleic acid molecules encoding antibodies comprised in the compositions of the invention

[0143] Examples of full length light chain nucleotide sequences optimized for expression in a mammalian cell are shown in SEQ ID NOs: 161-165 and 171-175. Examples of full length heavy chain nucleotide sequences optimized for expression in a mammalian cell are shown in SEQ ID NOs: 166-170 and 176-180.

[0144] The nucleic acids may be present in whole cells, in a cell lysate, or may be nucleic acids in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered

substantially pure" when purified away from other cellular components or other contaminants, *e.g.* other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. See, F. Ausubel, et al., ed. 1987 Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York. Nucleic acids can be obtained using standard molecular biology techniques. For antibodies expressed by hybridomas (*e.g.* hybridomas prepared from transgenic mice carrying human immunoglobulin genes as described further below), cDNAs encoding the light and heavy chains of the antibody made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques. For antibodies obtained from an immunoglobulin gene library (*e.g.* using phage display techniques), nucleic acid encoding the antibody can be recovered from various phage clones that are members of the library. Once DNA fragments encoding V_H and V_L segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to an scFv gene. In these manipulations, a V_L- or V_H-encoding DNA fragment is operatively linked to another DNA molecule, or to a fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked", as used in this context, is intended to mean that the two DNA fragments are joined in a functional manner, for example, such that the amino acid sequences encoded by the two DNA fragments remain in-frame, or such that the protein is expressed under control of a desired promoter.

[0145] The isolated DNA encoding the V_H region can be converted to a full-length heavy chain gene by operatively linking the V_H-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see *e.g.* Kabat, E. A., *et al.* [*supra*]) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region. The heavy chain contstant region can be selected among IgG1 isotypes. For a Fab fragment heavy chain gene, the V_H-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

[0146] The isolated DNA encoding the V_L region can be converted to a full-length light chain gene (as well as to a Fab light chain gene) by operatively linking the V_L-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see *e.g.* Kabat, E. A., *et al.* [*supra*]) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or a lambda constant region. To create an scFv gene, the V_H- and V_L-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.* encoding the amino acid sequence (Gly4 -Ser)$_3$, such that the V_H and V_L sequences can be expressed as a contiguous single-chain protein, with the V_L and V_H regions joined by the flexible linker (see *e.g.* Bird et al., 1988 Science 242:423-426; Huston

et al., 1988 Proc. Natl. Acad. Sci. USA 85:5879-5883; McCafferty et al., 1990 Nature 348:552-554).

Generation of monoclonal antibodies

[0147] Monoclonal antibodies (mAbs) can be produced by a variety of techniques, including conventional monoclonal antibody methodology *e.g.* the standard somatic cell hybridization technique of Kohler and Milstein (1975 Nature 256: 495). Many techniques for producing monoclonal antibody can be employed *e.g.* viral or oncogenic transformation of B lymphocytes.

[0148] An animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a well established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are known in the art. Fusion partners (*e.g.* murine myeloma cells) and fusion procedures are also known.

[0149] Chimeric or humanized antibodies comprised in the compositions of the present invention can be prepared based on the sequence of a murine monoclonal antibody prepared as described above. DNA encoding the heavy and light chain immunoglobulins can be obtained from the murine hybridoma of interest and engineered to contain non-murine (*e.g.* human) immunoglobulin sequences using standard molecular biology techniques. For example, to create a chimeric antibody, the murine variable regions can be linked to human constant regions using methods known in the art (see *e.g.* US4,816,567). To create a humanized antibody, the murine CDR regions can be inserted into a human framework using methods known in the art (see *e.g.* U.S. Patent No. 5225539; 5530101; 5585089; 5693762 and 6180370).

[0150] In a certain embodiment, the antibodies comprised in the compositions of the invention are human monoclonal antibodies. Such human monoclonal antibodies directed against ActRIIB can be generated using transgenic or transchromosomic mice carrying parts of the human immune system rather than the mouse system. These transgenic and transchromosomic mice include mice referred to herein as HuMAb mice and KM mice, respectively, and are collectively referred to herein as "human Ig mice."

[0151] The HuMAb mouse® (Medarex, Inc.) contains human immunoglobulin gene miniloci that encode un-rearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (see *e.g.* Lonberg, et al., 1994 Nature 368(6474): 856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal (Lonberg, N. *et al.,* 1994 [*supra*]; reviewed in Lonberg, N., 1994 Handbook of Experimental Pharmacology 113:49-101; Lonberg, N. and Huszar, D., 1995 Intern. Rev. Immunol.13: 65-93, and Harding, F. and Lonberg, N., 1995 Ann. N. Y. Acad. Sci. 764:536-546). The preparation and use of HuMAb mice, and the genomic

modifications carried by such mice, is further described in Taylor, L. et al., 1992 Nucleic Acids Research 20:6287-6295; Chen, J. et al., 1993 International Immunology 5: 647-656; Tuaillon et al., 1993 Proc. Natl. Acad. Sci. USA 94:3720-3724; Choi et al., 1993 Nature Genetics 4:117-123; Chen, J. et al., 1993 EMBO J. 12: 821-830; Tuaillon et al., 1994 J. Immunol. 152:2912-2920; Taylor, L. et al., 1994 International Immunology 579-591; and Fishwild, D. et al., 1996 Nature Biotechnology 14: 845-851. See further, U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; 5,770,429; and 5,545,807; as well as WO92/103918, WO93/12227, WO94/25585, WO97/113852, WO98/24884; WO99/45962; and WO01/14424.

[0152] In another embodiment, human antibodies comprised in the compositions of the invention can be raised using a mouse that carries human immunoglobulin sequences on transgenes and transchomosomes such as a mouse that carries a human heavy chain transgene and a human light chain transchromosome. Such mice, referred to herein as "KM mice", are described in detail in WO02/43478.

[0153] Still further, alternative transgenic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-ActRIIB antibodies of the invention. For example, an alternative transgenic system referred to as the Xenomouse (Abgenix, Inc.) can be used. Such mice are described in, *e.g.* U.S. Patent Nos. 5,939,598; 6,075,181; 6,114,598; 6, 150,584 and 6,162,963.

[0154] Moreover, alternative transchromosomic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-ActRIIB antibodies of the invention. For example, mice carrying both a human heavy chain transchromosome and a human light chain tranchromosome, referred to as "TC mice" can be used; such mice are described in Tomizuka et al., 2000 Proc. Natl. Acad. Sci. USA 97:722-727. Furthermore, cows carrying human heavy and light chain transchromosomes have been described in the art (Kuroiwa et al., 2002 Nature Biotechnology 20:889-894) and can be used to raise anti-ActRIIB antibodies.

[0155] Human recombinant antibodies comprised in the compositions of the invention can also be prepared using phage display methods for screening libraries of human immunoglobulin genes. Such phage display methods for isolating human antibodies are established in the art or described in the examples below. See for example: U.S. Patent Nos. 5,223,409; 5,403,484; 5,571,698; 5,427,908; 5,580,717; 5,969,108; 6,172,197; 5,885,793; 6,521,404; 6,544,731; 6,555,313; 6,582,915 and 6,593,081.

[0156] Human monoclonal antibodies comprised in the compositions of the invention can also be prepared using SCID mice into which human immune cells have been reconstituted such that a human antibody response can be generated upon immunization. Such mice are described in, for example, U.S. Patent Nos. 5,476,996 and 5,698,767.

Generation of hybridomas producing human monoclonal antibodies

[0157] To generate hybridomas producing human monoclonal antibodies comprised in the compositions of the invention, splenocytes and/or lymph node cells from immunized mice can be isolated and fused to an appropriate immortalized cell line, such as a mouse myeloma cell line. The resulting hybridomas can be screened for the production of antigen-specific antibodies. For example, single cell suspensions of splenic lymphocytes from immunized mice can be fused to one-sixth the number of P3X63-Ag8.653 nonsecreting mouse myeloma cells (ATCC, CRL 1580) with 50% PEG. Cells are plated at approximately 2 x 145 in flat bottom microtiter plates, followed by a two week incubation in selective medium containing 20% fetal Clone Serum, 18% "653" conditioned media, 5% origen (IGEN), 4 mM L-glutamine, 1 mM sodium pyruvate, 5mM HEPES, 0:055 mM 2-mercaptoethanol, 50 units/ml penicillin, 50 mg/ml streptomycin, 50 mg/ml gentamycin and 1X HAT (Sigma; the HAT is added 24 hours after the fusion). After approximately two weeks, cells can be cultured in medium in which the HAT is replaced with HT. Individual wells can then be screened by ELISA for human monoclonal IgM and IgG antibodies. Once extensive hybridoma growth occurs, medium can be observed usually after 10-14 days. The antibody secreting hybridomas can be replated, screened again, and if still positive for human IgG, the monoclonal antibodies can be subcloned at least twice by limiting dilution. The stable subclones can then be cultured *in vitro* to generate small amounts of antibody in tissue culture medium for characterization.

[0158] To purify human monoclonal antibodies, selected hybridomas can be grown in two-liter spinner-flasks for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia). Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD_{280} using 1.43 extinction coefficient. The monoclonal antibodies can be aliquoted and stored at -80°C.

Generation of transfectomas producing monoclonal antibodies

[0159] Antibodies comprised in the compositions of the invention also can be produced in a host cell transfectoma using, for example, a combination of recombinant DNA techniques and gene transfection methods as is well known in the art (*e.g.* Morrison, S. (1985) Science 229:1202).

[0160] For example, to express the antibodies, or antibody fragments thereof, DNAs encoding partial or full-length light and heavy chains, can be obtained by standard molecular biology techniques (*e.g.* PCR amplification or cDNA cloning using a hybridoma that expresses the antibody of interest) and the DNAs can be inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such

that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (*e.g.* ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). The light and heavy chain variable regions of the antibodies described herein can be used to create full-length antibody genes of any antibody isotype by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype such that the V_H segment is operatively linked to the CH segment(s) within the vector and the V_L segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.* a signal peptide from a non-immunoglobulin protein).

[0161] In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.* polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel (Gene Expression Technology. Methods in Enzymology 185, Academic Press, San Diego, CA 1990). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences, may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.* Regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus (*e.g.* the adenovirus major late promoter (AdMLP)), and polyoma. Alternatively, nonviral regulatory sequences may be used, such as the ubiquitin promoter or P-globin promoter. Still further, regulatory elements composed of sequences from different sources, such as the SRa promoter system, which contains sequences from the SV40 early promoter and the long terminal repeat of human T cell leukemia virus type 1 (Takebe, Y. et al., 1988 Mol. Cell. Biol. 8:466-472).

[0162] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors may carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.* origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, *e.g.* U.S. Patent Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or

methotrexate, on a host cell into which the vector has been introduced. Selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr-host cells with methotrexate selection/amplification) and the neo gene (for G418 selection). For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.* electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. It is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells. Expression of antibodies in eukaryotic cells, in particular mammalian host cells, is discussed because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Prokaryotic expression of antibody genes has been reported to be ineffective for production of high yields of active antibody (Boss, M. A. and Wood, C. R., 1985 Immunology Today 6:12-13).

[0163] Mammalian host cells for expressing the recombinant antibodies comprised in the compositions of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described Urlaub and Chasin, 1980 Proc. Natl. Acad. Sci. USA 77:4216-4220 used with a DH FR selectable marker, *e.g.* as described in R.J. Kaufman and P.A. Sharp, 1982 Mol. Biol. 159:601-621), NSO myeloma cells, COS cells and SP2 cells. In one embodiment the host cells are CHO K1PD cells. In particular, for use with NSO myeloma cells, another expression system is the GS gene expression system shown in WO87/04462, WO89/01036 and EP 338,841. Mammalian host cells for expressing the recombinant antibodies comprised in the compositions of the invention include mammalian cell lines deficient for FUT8 gene expression, for example as described in US6,946,292B2. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Immunoconjugates

[0164] In another aspect, the present invention features compositions comprising an anti-ActRIIB antibody, or a fragment thereof, conjugated to a therapeutic moiety, such as a cytotoxin, a drug (*e.g.* an immunosuppressant) or a radiotoxin. Such conjugates are referred to herein as "immunoconjugates". Immunoconjugates that include one or more cytotoxins are referred to as "immunotoxins." A cytotoxin or cytotoxic agent includes any agent that is detrimental to (*e.g.* kills) cells.

[0165] Cytotoxins can be conjugated to antibodies of the invention using linker technology available in the art. Examples of linker types that have been used to conjugate a cytotoxin to

an antibody include, but are not limited to, hydrazones, thioethers, esters, disulfides and peptide-containing linkers. A linker can be chosen that is, for example, susceptible to cleavage by low pH within the lysosomal compartment or susceptible to cleavage by proteases, such as proteases preferentially expressed in tumor tissue such as cathepsins (*e.g.* cathepsins B, C, D).

[0166] For further discussion of types of cytotoxins, linkers and methods for conjugating therapeutic agents to antibodies, see also Saito, G. et al., 2003 Adv. Drug Deliv. Rev. 55:199-215; Trail, P.A. et al., 2003 Cancer Immunol. Immunother. 52:328-337; Payne, G. 2003 Cancer Cell 3:207-212; Allen, T.M., 2002 Nat. Rev. Cancer 2:750-763; Pastan, I. and Kreitman, R. J., 2002 Curr. Opin. Investig. Drugs 3:1089-1091; Senter, P.D. and Springer, C.J., 2001 Adv. Drug Deliv. Rev. 53:247-264.

[0167] Antibodies comprised in the compositions of the present invention also can be conjugated to a radioactive isotope to generate cytotoxic radiopharmaceuticals, also referred to as radioimmunoconjugates. Examples of radioactive isotopes that can be conjugated to antibodies for use diagnostically or therapeutically include, but are not limited to, iodine^{131}, indium^{111}, yttrium^{90}, and lutetium^{177}. Methods for preparing radioimmunconjugates are established in the art. Examples of radioimmunoconjugates are commercially available, including Zevalin™ (DEC Pharmaceuticals) and Bexxar™ (Corixa Pharmaceuticals), and similar methods can be used to prepare radioimmunoconjugates using the antibodies of the invention.

[0168] The antibody conjugates comprised in the compositions of the invention can be used to modify a given biological response, and the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, an enzymatically active toxin, or active fragment thereof, such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor or interferon-γ; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[0169] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.* Amon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Inmunol. Rev., 62:119-58 (1982).

Pharmaceutical compositions

[0170] In another aspect, the present invention provides a composition, *e.g.* a pharmaceutical composition, containing one or a combination of the above described antibodies/monoclonal antibodies, or antigen-binding portion(s) thereof, formulated together with a pharmaceutically acceptable carrier. Such compositions may include one or a combination of (*e.g.* two or more different) the described antibodies, or immunoconjugates or bispecific molecules. For example, a pharmaceutical composition of the invention can comprise a combination of antibodies that bind to different epitopes on the target antigen or that have complementary activities.

[0171] Pharmaceutical compositions of the invention also can be administered in combination therapy, *i.e.* combined with other agents. For example, the combination therapy can include an anti-ActRIIB antibody of the present invention combined with at least one other muscle mass/strength increasing agent, for example, IGF-1, IGF-2 or variants of IGF-1 or IGF-2, an anti-myostatin antibody, a myostatin propeptide, a myostatin decoy protein that binds ActRIIB but does not activate it, a beta 2 agonist, a Ghrelin agonist, a SARM, GH agonists/mimetics or follistatin. Examples of therapeutic agents that can be used in combination therapy are described in greater detail below in the section on uses of the antibodies of the invention.

[0172] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The carrier should be suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.* by injection or infusion). Depending on the route of administration, the active compound, *i.e.* antibody, immunoconjuage, or bispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

[0173] The pharmaceutical compositions of the invention may include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see *e.g.* Berge, S.M., et al., 1977 J. Pharm. Sci. 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and di-carboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

[0174] A pharmaceutical composition of the invention also may include a pharmaceutically acceptable anti-oxidant. Examples of pharmaceutically acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[0175] Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0176] These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, *supra,* and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as, aluminum monostearate and gelatin.

[0177] Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[0178] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, one can include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption for example, monostearate salts and gelatin.

[0179] Sterile injectable solutions can be prepared by incorporating the active compound in the

required amount in an appropriate solvent with one or a combination of agents enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other agents from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active agent plus any additional desired agent from a previously sterile-filtered solution thereof.

[0180] The amount of active agent which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active agent which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 0.01 per cent to about ninety-nine percent of active agent, from about 0.1 per cent to about 70 per cent, or from about 1 percent to about 30 percent of active agent in combination with a pharmaceutically acceptable carrier.

[0181] Dosage regimens are adjusted to provide the optimum desired response (*e.g.* a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0182] For administration of the antibody comprising composition, the antibody dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 30 mg/kg, of the host body weight. For example dosages can be about 0.3 mg/kg body weight, about 1 mg/kg body weight, about 3 mg/kg body weight, about 5 mg/kg body weight or about 10 mg/kg body weight or about 30 mg/kg body weight within the ranges of about 1-10 mg/kg or about 3-7 mg/kg. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. Alternatively, the antibody may be administered about once a year or once only. Such administration may be carried out intraveneously or subcutaneously. Dosage regimens for an anti-ActRIIB antibody of the invention include 1 mg/kg body weight or 3 mg/kg body weight by intravenous administration, with the antibody being given using one of the following dosing schedules: every four weeks for six dosages, then every three months; every three weeks; 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks.

[0183] The dosage should be one that causes an increase in BAT while not increasing red blood cell levels in a subject. In some methods, two or more monoclonal antibodies with different binding specificities are comprised in the compositions of the invention and, thus, administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, monthly, every three months, every six months or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1-1000 µg/ml and in some methods about 25-300 µg/ml. For example, an ActRIIB antibody of the invention could be co-administered with an anti-myostatin antibody.

[0184] Alternatively, the composition can be a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human antibodies show the longest half-life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated or until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

[0185] Actual dosage levels of the active agents in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active agent which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[0186] Administration of a "therapeutically effective dosage" of an anti-ActRIIB antibody comprised in the compositions of the invention can result in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction *i.e.* an increase in muscle mass and/or strength.

[0187] A composition of the present invention can be administered by one or more routes of administration using one or more of a variety of methods known in the art. As will be

appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Routes of administration for antibodies of the invention include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrastemal injection and infusion. In one embodiment the antibody comprising composition is administered intravenously. In another embodiment the antibody is administered subcutaneously. Alternatively, an antibody comprising composition of the invention can be administered by a nonparenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

[0188] The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.* Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0189] Therapeutic compositions can be administered with medical devices known in the art. For example, in one embodiment, a therapeutic composition of the invention can be administered with a needleless hypodermic injection device, such as the devices shown in U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824 or 4,596,556. Examples of well known implants and modules useful in the present invention include: U.S. Patent No. 4,487,603, which shows an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,486,194, which shows a therapeutic device for administering medicants through the skin; U.S. Patent No. 4,447,233, which shows a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which shows a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which shows an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which shows an osmotic drug delivery system. Many other such implants, delivery systems, and modules are known to those skilled in the art and include those made by MicroCHIPS™ (Bedford, MA).

[0190] In certain embodiments, the human monoclonal antibodies comprising composition of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.* U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties

which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, *e.g.* V.V. Ranade, 1989 J. Clin Pharmacol. 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.* U.S. Patent 5,416,016); mannosides (Umezawa et al., 1988 Biochem. Biophys. Res. Commun. 153:1038); antibodies (P.G. Bloeman et al., 1995 FEBS Lett. 357:140; M. Owais et al., 1995 Antimicrob. Agents Chernother. 39:180); surfactant protein A receptor (Briscoe et al., 1995 Am. J. Physiol.1233:134); p120 (Schreier et al., 1994 J. Biol. Chem. 269:9090); see also K. Keinanen; M.L. Laukkanen, 1994 FEBSLett. 346:123; J.J. Killion; I.J. Fidler, 1994 Imrnunomethods 4:273.

Uses and methods of the invention

[0191] The invention also relates to the use of an anti-ActRIIB antibody as defined in the present claims in the manufacture of a medicament for the treatment of a metabolic disorder, particularly obesity, Type 2 Diabetes, Metabolic Syndrome, lipodystrophy, impaired glucose tolerance, elevated plasma insulin concentrations, insulin resistance, dyslipidemia, hyperglycemia, hyperlipidemia, hypertension, cardiovascular disease or respiratory problems or for decreasing the average plasma glucose concentration, controlling the glucose homeostasis or the insulin sensitivity.

[0192] The methods are particularly suitable for treating, preventing or ameliorating metabolic disorders.

[0193] The disclosure also relates to the methods of treating a subject suffering from a metabolic disorder and a musculoskeletal diseases or disorders, such as muscle atrophy. Muscle atrophy can be obesity-associated sarcopenia, sarcopenia, or diabetes-associated muscle atrophy.

[0194] There are many causes of muscle atrophy, including as a result of treatment with a glucocorticoid such as cortisol, dexamethasone, betamethasone, prednisone, methylprednisolone, or prednisolone. The muscle atrophy can also be a result of denervation due to nerve trauma or a result of degenerative, metabolic, or inflammatory neuropathy (*e.g.*, Guillian-Barré syndrome, peripheral neuropathy, or exposure to environmental toxins or drugs). In addition, the muscle atrophy can be a result of myopathy, such as myotonia; a congential myopathy, including nemalene myopathy, multi/minicore myopathy and myotubular (centronuclear) myopathy; mitochondrial myopathy; familial periodic paralysis; inflammatory myopathy; metabolic myopathy, such as caused by a glycogen or lipid storage disease; dermatomyositisis; polymyositis; inclusion body myositis; myositis ossificans; rhabdomyolysis and myoglobinurias. Other conditions leading to musculoskeletal diseases or muscle atrophy are described in detail above. The treatment results may be complete, e.g., the total absence of a metabolic disorder. The results may also be partial, such that the peculiarity of the metabolic disorder in a subject is statistically significantly less pronounced than had the subject not received a composition of the present invention. Partial treatment results may be a decrease in severity of disease symptoms, an increase in frequency and duration of disease

symptom-free periods, or a prevention of impairment or disability due to the disease affliction. An age-related condition as referred to herein may begin at the age of 50 years or older (*i.e.* 60, 70, 80 or older).

[0195] In one embodiment, a patient may be pre-treated with an anti-ActRIIB antibody or a compostion according to the present claims prior to an anticipated period of enforced rest/inactivity. Such a period may occur when a patient is admitted to hospital, for example for surgery to the hip or leg. The inactivity may be localised, such as by casting of a broken limb or joint, or by administration of a paralytic agent.

[0196] In a further embodiment, the patient may be one who has not responded to previous treatments. For example, the patient may not have responded to treatment with IGF-1, IGF-2 or variants of IGF-1 or IGF-2, an anti-myostatin antibody, a myostatin propeptide, a myostatin decoy protein that binds ActRIIB but does not activate it, a beta 2 agonist, a Ghrelin agonist, a SARM, GH agonists/mimetics or follistatin. A simple way of measuring a patient's response to treatment may be timing how long it takes for a patient to climb a known height of stairs and comparing the results both before and after treatment.

[0197] The ActRIIB antibodies may be administered as the sole active agent or in conjunction with, *e.g.* as an adjuvant to or in combination to, other drugs *e.g.* IGF-1, IGF-2 or variants of IGF-1 or IGF-2, an anti-myostatin antibody, a myostatin propeptide, a myostatin decoy protein that binds ActRIIB but does not activate it, a beta 2 agonist, a Ghrelin agonist, a SARM, GH agonists/mimetics or follistatin. For example, the antibodies of the invention may be used in combination with an IGF-1 mimetic as disclosed in WO2007/146689.

[0198] In accordance with the foregoing the present invention provides in a yet further aspect:
A method or use as defined above comprising co-administration, *e.g.* concomitantly or in sequence, of a therapeutically effective amount of an ActRIIB antibody , and at least one second drug substance, said second drug substance being IGF-1, IGF-2 or variants of IGF-1 or IGF-2, an anti-myostatin antibody, a myostatin propeptide, a myostatin decoy protein that binds ActRIIB but does not activate it, a beta 2 agonist, a Ghrelin agonist, a SARM, GH agonists/mimetics or follistatin.

SEQUENCES

[0199]

Table 1: sequence listing

SEQ ID NO	Ab region	Sequence
SEQ ID NO1	HCDR1	GYTFTSSYIN

SEQ ID NO	Ab region	Sequence
SEQ ID NO2	HCDR1	GYTFTSSYIN
SEQ ID NO3	HCDR1	GYTFTSSYIN
SEQ ID NO4	HCDR1	GYTFTSSYIN
SEQ ID NO5	HCDR1	GYTFTSSYIN
SEQ ID NO6	HCDR1	GYTFTSSYIN
SEQ ID NO7	HCDR1	GYTFTSSYIN
SEQ ID NO8	HCDR1	GYTFTSSYIN
SEQ ID NO9	HCDR1	GYTFTSSYIN
SEQ ID NO10	HCDR1	GYTFTSSYIN
SEQ ID NO11	HCDR1	GYTFTSSYIN
SEQ ID NO12	HCDR1	GYTFTSSYIN
SEQ ID NO13	HCDR1	GYTFTSSYIN
SEQ ID NO14	HCDR1	GYTFTSSYIN
SEQ ID NO15	HCDR2	TINPVSGNTSYAQKFQG
SEQ	HCDR2	TINPVSGNTSYAQKFQG

SEQ ID NO	Ab region	Sequence
ID NO16		
SEQ ID NO17	HCDR2	TINPVSGNTSYAQKFQG
SEQ ID NO18	HCDR2	TINPVSGNTSYAQKFQG
SEQ ID NO19	HCDR2	MINAPIGTTRYAQKFQG
SEQ ID NO20	HCDR2	QINAASGMTRYAQKFQG
SEQ ID NO21	HCDR2	MINAPIGTTRYAQKFQG
SEQ ID NO22	HCDR2	TINPVSGNTRYAQKFQG
SEQ ID NO23	HCDR2	TINPVSGSTSYAQKFQG
SEQ ID NO24	HCDR2	QINAASGMTRYAQKFQG
SEQ ID NO25	HCDR2	NINAAAGITLYAQKFQG
SEQ ID NO26	HCDR2	TINPPTGGTYYAQKFQG
SEQ ID NO27	HCDR2	GINPPAGTTSYAQKFQG
SEQ ID NO28	HCDR2	NINPATGHADYAQKFQG
SEQ ID NO29	HCDR3	GGWFDY
SEQ	HCDR3	GGWFDY

SEQ ID NO	Ab region	Sequence
ID NO30		
SEQ ID NO31	HCDR3	GGWFDY
SEQ ID NO32	HCDR3	GGWFDY
SEQ ID NO33	HCDR3	GGWFDY
SEQ ID NO34	HCDR3	GGWFDY
SEQ ID NO35	HCDR3	GGWFDY
SEQ ID NO36	HCDR3	GGWFDY
SEQ ID NO37	HCDR3	GGWFDY
SEQ ID NO38	HCDR3	GGWFDY
SEQ ID NO39	HCDR3	GGWFDY
SEQ ID NO40	HCDR3	GGWFDY
SEQ ID NO41	HCDR3	GGWFDY
SEQ ID NO42	HCDR3	GGWFDY
SEQ ID NO43	LCDR1	TGTSSDVGSYNYVN
SEQ ID	LCDR1	TGTSSDVGSYNYVN

SEQ ID NO	Ab region	Sequence
NO44		
SEQ ID NO45	LCDR1	TGTSSDVGSYNYVN
SEQ ID NO46	LCDR1	TGTSSDVGSYNYVN
SEQ ID NO47	LCDR1	TGTSSDVGSYNYVN
SEQ ID NO48	LCDR1	TGTSSDVGSYNYVN
SEQ ID NO49	LCDR1	TGTSSDVGSYNYVN
SEQ ID NO50	LCDR1	TGTSSDVGSYNYVN
SEQ ID NO51	LCDR1	TGTSSDVGSYNYVN
SEQ ID NO52	LCDR1	TGTSSDVGSYNYVN
SEQ ID NO53	LCDR1	TGTSSDVGSYNYVN
SEQ ID NO54	LCDR1	TGTSSDVGSYNYVN
SEQ ID NO55	LCDR1	TGTSSDVGSYNYVN
SEQ ID NO56	LCDR1	TGTSSDVGSYNYVN
SEQ ID NO57	LDCR2	LMIYGVSKRPS
SEQ ID	LDCR2	LMIYGVSKRPS

SEQ ID NO	Ab region	Sequence
NO58		
SEQ ID NO59	LDCR2	LMIYGVSKRPS
SEQ ID NO60	LDCR2	LMIYGVSKRPS
SEQ ID NO61	LDCR2	LMIYGVSKRPS
SEQ ID NO62	LDCR2	LMIYGVSKRPS
SEQ ID NO63	LDCR2	LMIYGVSKRPS
SEQ ID NO64	LDCR2	LMIYGVSKRPS
SEQ ID NO65	LDCR2	LMIYGVSKRPS
SEQ ID NO66	LDCR2	LMIYGVSKRPS
SEQ ID NO67	LDCR2	LMIYGVSKRPS
SEQ ID NO68	LDCR2	LMIYGVSKRPS
SEQ ID NO69	LDCR2	LMIYGVSKRPS
SEQ ID NO70	LDCR2	LMIYGVSKRPS
SEQ ID NO71	LCDR3	QAWTSKMAG
SEQ ID NO72	LCDR3	SSYTRMGHP

SEQ ID NO	Ab region	Sequence
SEQ ID NO73	LCDR3	ATYGKGVTPP
SEQ ID NO74	LCDR3	GTFAGGSYYG
SEQ ID NO75	LCDR3	QAWTSKMAG
SEQ ID NO76	LCDR3	QAWTSKMAG
SEQ ID NO77	LCDR3	GTFAGGSYYG
SEQ ID NO78	LCDR3	GTFAGGSYYG
SEQ ID NO79	LCDR3	GTFAGGSYYG
SEQ ID NO80	LCDR3	GTFAGGSYYG
SEQ ID NO81	LCDR3	GTFAGGSYYG
SEQ ID NO82	LCDR3	GTFAGGSYYG
SEQ ID NO83	LCDR3	GTFAGGSYYG
SEQ ID NO84	LCDR3	GTFAGGSYYG
SEQ ID NO85	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCQAWTSKMAGVFGGGTKLTVLGQ
SEQ ID	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV

SEQ ID NO	Ab region	Sequence
NO86		SNRFSGSKSGNTASLTISGLQAEDEADYYCSSYTRMGHPVFGGGTKLTVLGQ
SEQ ID NO87	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCATYGKGVTPPVFGGGTKLTVLGQ
SEQ ID NO88	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQ
SEQ ID NO89	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCQAWTSKMAGVFGGGTKLTVLGQ
SEQ ID NO90	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCQAWTSKMAGVFGGGTKLTVLGQ
SEQ ID NO91	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQ
SEQ ID NO92	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQ
SEQ ID NO93	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQ
SEQ ID NO94	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQ
SEQ ID NO95	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQ
SEQ ID NO96	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQ
SEQ ID NO97	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQ
SEQ ID	VL	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV

SEQ ID NO	Ab region	Sequence
NO98		SNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQ
SEQ ID NO99	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGTINPVSGNT SYAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID NO100	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGTINPVSGNT SYAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID NO101	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGTINPVSGNT SYAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID NO102	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGTINPVSGNT SYAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID NO103	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGMINAPIGTTR YAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID NO104	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGQINAASGMT RYAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID NO105	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGMINAPIGTTR YAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID NO106	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGTINPVSGNT RYAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID NO107	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGTINPVSGST SYAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID NO108	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGQINAASGMT RYAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID NO109	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGNINAAAGITL YAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGTINPPTGGT

SEQ ID NO	Ab region	Sequence
NO110		YYAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID NO111	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGGINPPAGTT SYAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID NO112	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGNINPATGHA DYAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQGTLVTVSS
SEQ ID NO113	DNA VL	GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACC AGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCT CAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACC ATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCCAGGCTTGGACTTCT AAGATGGCTGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ ID NO114	DNA VL	GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACC AGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCT CAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACC ATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCTCTTCTTATACTCGTA TGGGTCATCCTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ ID NO115	DNA VL	GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACC AGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCT CAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACC ATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGCTACTTATGGTAAG GGTGTTACTCCTCCTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ ID NO116	DNA VL	GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACC AGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCT CAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACC ATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGGT GGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ ID NO117	DNA VL	GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACC AGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCT CAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACC ATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCCAGGCTTGGACTTCT AAGATGGCTGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ	DNA VL	

SEQ ID NO	Ab region	Sequence
ID NO118		GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACC AGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCT CAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACC ATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCCAGGCTTGGACTTCT AAGATGGCTGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ ID NO119	DNA VL	GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACC AGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCT CAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACC ATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGGT GGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ ID NO120	DNA VL	GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACC AGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCT CAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACC ATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGGT GGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ ID NO121	DNA VL	GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACC AGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCT CAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACC ATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGGT GGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ ID NO122	DNA VL	GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACC AGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCT CAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACC ATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGGT GGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ ID NO123	DNA VL	GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACC AGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCT CAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACC ATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGGT GGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ ID NO124	DNA VL	GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACTGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACCA GCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCTC AGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACCAT TAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGGTGG

SEQ ID NO	Ab region	Sequence
		TTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ ID NO125	DNA VL	GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACC AGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCT CAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACC ATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGGT GGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ ID NO126	DNA VL	GATATCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTACC ATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTACC AGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCCT CAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGACC ATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGGT GGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTTCTTGGCCAG
SEQ ID NO127	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCACTATCAATCCGGTTTCTGGCAATA CGTCTTACGCGCAGAAGTTTCAGGGCCGGGTGACCATGACCCGTGATACCAGCATTA GCACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATT GCGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCT CA
SEQ ID NO128	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCACTATCAATCCGGTTTCTGGCAATA CGTCTTACGCGCAGAAGTTTCAGGGCCGGGTGACCATGACCCGTGATACCAGCATTA GCACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATT GCGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCT CA
SEQ ID NO129	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCACTATCAATCCGGTTTCTGGCAATA CGTCTTACGCGCAGAAGTTTCAGGGCCGGGTGACCATGACCCGTGATACCAGCATTA GCACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATT GCGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCT CA
SEQ ID NO130	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCACTATCAATCCGGTTTCTGGCAATA CGTCTTACGCGCAGAAGTTTCAGGGCCGGGTGACCATGACCCGTGATACCAGCATTA GCACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATT GCGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCT CA

SEQ ID NO	Ab region	Sequence
		CA
SEQ ID NO131	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCATGATTAATGCTCCTATTGGTACTA CTCGTTATGCTCAGAAGTTTCAGGGTCGGGTGACCATGACCCGTGATACCAGCATTA GCACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATT GCGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCT CA
SEQ ID NO132	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCCAGATTAATGCTGCTTCTGGTATGA CTCGTTATGCTCAGAAGTTTCAGGGTCGGGTGACCATGACCCGTGATACCAGCATTA GCACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATT GCGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCT CA
SEQ ID NO133	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCATGATTAATGCTCCTATTGGTACTA CTCGTTATGCTCAGAAGTTTCAGGGTCGGGTGACCATGACCCGTGATACCAGCATTA GCACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATT GCGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCT CA
SEQ ID NO134	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCACTATCAATCCGGTTTCTGGCAATA CGCGTTACGCGCAGAAGTTTCAGGGCCGGGTGACCATGACCCGTGATACCAGCATTA GCACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATT GCGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCT CA
SEQ ID NO135	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCACTATCAATCCGGTTTCTGGCTCTA CGTCTTACGCGCAGAAGTTTCAGGGCCGGGTGACCATGACCCGTGATACCAGCATTA GCACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATT GCGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCT CA
SEQ ID NO136	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCCAGATTAATGCTGCTTCTGGTATGA CTCGTTATGCTCAGAAGTTTCAGGGTCGGGTCACCATGACCCGTGATACCAGCATTA GCACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATT

SEQ ID NO	Ab region	Sequence
		GCGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCT CA
SEQ ID NO137	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCAATATTAATGCTGCTGCTGGTATTA CTCTTTATGCTCAGAAGTTTCAGGGTCGGGTCACCATGACCCGTGATACCAGCATTAG CACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATTG CGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCTC A
SEQ ID NO138	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCACTATTAATCCTCCTACTGGAGGTA CTTATTATGCTCAGAAGTTTCAGGGTCGGGTGACCATGACCCGTGATACCAGCATTAG CACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATTG CGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCTC A
SEQ ID NO139	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCGGTATTAATCCTCCTGCTGGTACTA CTTCTTATGCTCAGAAGTTTCAGGGTCGGGTCACCATGACCCGTGATACCAGCATTAG CACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATTG CGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCTC A
SEQ ID NO140	DNA VH	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCAATATTAATCCTGCTACTGGTCATG CTGATTATGCTCAGAAGTTTCAGGGTCGGGTGACCATGACCCGTGATACCAGCATTA GCACCGCGTATATGGAACTGAGCAGCCTGCGTAGCGAAGATACGGCCGTGTATTATT GCGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCT CA
SEQ ID NO141	Light Chain	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSG VSNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQPKAAP SVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO142	Light Chain	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSG VSNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQPKAAP SVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
SEQ	Light Chain	

SEQ ID NO	Ab region	Sequence
ID NO143		QSALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSG VSNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQPKAAP SVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO144	Light Chain	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSG VSNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQPKAAP
		SVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO145	Light Chain	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSG VSNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQPKAAP SVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO146	Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGTINPVSGST SYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGWFDYWGQGTLVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO147	Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGQINAASGMT RYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGWFDYWGQGTLVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO148	Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGNINAAAGITL YAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGWFDYWGQGTLVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID	Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGGINPPAGTT

SEQ ID NO	Ab region	Sequence
NO149		SYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGWFDYWGQGTLVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO150	Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGNINPATGHA DYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGWFDYWGQGTLVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO151	Light Chain	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSG VSNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQPKAAP SVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO152	Light Chain	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSG VSNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQPKAAP SVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO153	Light Chain	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSG VSNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQPKAAP SVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO154	Light Chain	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSG VSNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQPKAAP SVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO155	Light Chain	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSG VSNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQPKAAP SVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO156	Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGTINPVSGST SYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGWFDYWGQGTLVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG

SEQ ID NO	Ab region	Sequence
		LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFR VVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO157	Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGQINAASGMT RYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGWFDYWGQGTLVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFR VVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO158	Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGNINAAAGITL YAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGWFDYWGQGTLVTVSSAS TKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NQ159	Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGGINPPAGTT SYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGWFDYWGQGTLVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFR VVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO160	Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSSYINWVRQAPGQGLEWMGNINPATGHA DYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGWFDYWGQGTLVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFR VVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO161	DNA Light Chain	CAGAGCGCCCTGACCCAGCCCGCCAGCGTGTCCGGCAGCCCAGGCCAGTCTATCAC AATCAGCTGCACCGGCACCTCCAGCGACGTGGGCAGCTACAACTACGTGAACTGGTA TCAGCAGCACCCCGGCAAGGCCCCCAAGCTGATGATCTACGGCGTGAGCAAGAGGC CCAGCGGCGTGTCCAACAGGTTCAGCGGCAGCAAGAGCGGCAACACCGCCAGCCTG ACAATCAGTGGGCTGCAGGCTGAGGACGAGGCCGACTACTACTGCGGCACCTTTGC CGGCGGATCATACTACGGCGTGTTCGGCGGAGGGACCAAGCTGACCGTGCTGGGCC

SEQ ID NO	Ab region	Sequence
		AGCCTAAGGCTGCCCCCAGCGTGACCCTGTTCCCCCCCAGCAGCGAGGAGCTGCAG GCCAACAAGGCCACCCTGGTGTGCCTGATCAGCGACTTCTACCCAGGCGCCGTGAC CGTGGCCTGGAAGGCCGACAGCAGCCCCGTGAAGGCCGGCGTGGAGACCACCACC CCCAGCAAGCAGAGCAACAACAAGTACGCCGCCAGCAGCTACCTGAGCCTGACCCC CGAGCAGTGGAAGAGCCACAGGTCCTACAGCTGCCAGGTGACCCACGAGGGCAGCA CCGTGGAAAAGACCGTGGCCCCAACCGAGTGCAGC
SEQ ID NO162	DNA Light Chain	CAGAGCGCCCTGACCCAGCCCGCCAGCGTGTCCGGCAGCCCAGGCCAGTCTATCAC AATCAGCTGCACCGGCACCTCCAGCGACGTGGGCAGCTACAACTACGTGAACTGGTA TCAGCAGCACCCCGGCAAGGCCCCCAAGCTGATGATCTACGGCGTGAGCAAGAGGC CCAGCGGCGTGTCCAACAGGTTCAGCGGCAGCAAGAGCGGCAACACCGCCAGCCTG ACAATCAGTGGGCTGCAGGCTGAGGACGAGGCCGACTACTACTGCGGCACCTTTGC CGGCGGATCATACTACGGCGTGTTCGGCGGAGGGACCAAGCTGACCGTGCTGGGCC AGCCTAAGGCTGCCCCCAGCGTGACCCTGTTCCCCCCCAGCAGCGAGGAGCTGCAG GCCAACAAGGCCACCCTGGTGTGCCTGATCAGCGACTTCTACCCAGGCGCCGTGAC CGTGGCCTGGAAGGCCGACAGCAGCCCCGTGAAGGCCGGCGTGGAGACCACCACC CCCAGCAAGCAGAGCAACAACAAGTACGCCGCCAGCAGCTACCTGAGCCTGACCCC CGAGCAGTGGAAGAGCCACAGGTCCTACAGCTGCCAGGTGACCCACGAGGGCAGCA CCGTGGAAAAGACCGTGGCCCCAACCGAGTGCAGC
SEQ ID NO163	DNA Light Chain	CAGAGCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTAC CATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTAC CAGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCC TCAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGAC CATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGG TGGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCAGCC CAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAA CAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGC CTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTCCA AACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCCTGAGCAGT GGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAG AAGACAGTGGCCCCTACAGAATGTTCA
SEQ ID NO164	DNA Light Chain	CAGAGCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTAC CATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTAC CAGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCC TCAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGAC CATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGG TGGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCAGCC
		CAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAA CAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGC CTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTCCA AACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCCTGAGCAGT GGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAG AAGACAGTGGCCCCTACAGAATGTTCA
SEQ ID	DNA Light Chain	CAGAGCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTAC

SEQ ID NO	Ab region	Sequence
NO165		CATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTAC CAGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCC TCAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGAC CATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGG TGGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCAGCC CAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAA CAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGC CTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTCCA AACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCCTGAGCAGT GGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAG AAGACAGTGGCCCCTACAGAATGTTCA
SEQ ID NO166	DNA Heavy Chain	CAGGTGCAGCTGGTGCAGAGCGGAGCTGAGGTGAAGAAGCCAGGCGCCAGCGTCAA GGTGTCCTGCAAGGCCAGCGGCTACACCTTCACCAGCAGCTACATCAACTGGGTCCG CCAGGCTCCTGGGCAGGGACTGGAGTGGATGGGCACCATCAACCCCGTGTCCGGCA GCACCAGCTACGCCCAGAAGTTCCAGGGCAGAGTCACCATGACCAGGGACACCAGC ATCAGCACCGCCTACATGGAGCTGTCCAGGCTGAGAAGCGACGACACCGCCGTGTA CTACTGCGCCAGGGGCGGCTGGTTCGACTACTGGGGCCAGGGCACCCTGGTGACCG TGTCCTCAGCTAGCACCAAGGGCCCCAGCGTGTTCCCCCTGGCCCCCAGCAGCAAG AGCACCTCCGGCGGCACAGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGA GCCCGTGACCGTGTCCTGGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTTCC CCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGTCCAGCGTGGTGACAGTGCCC AGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAAC ACCAAGGTGGACAAGAGAGTGGAGCCCAAGAGCTGCGACAAGACCCACACCTGCCC CCCCTGCCCAGCCCCCGAAGCTGCAGGCGGCCCTTCCGTGTTCCTGTTCCCCCCCA AGCCCAAGGACACCCTGATGATCAGCAGGACCCCCGAGGTGACCTGCGTGGTGGTG GACGTGAGCCACGAGGACCCAGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGA GGTGCACAACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACAGGG TGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGT GCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAAAAGACCATCAGCAAGGCCA AGGGCCAGCCACGGGAGCCCCAGGTGTACACCCTGCCCCCTTCTCGGGAGGAGATG ACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCC AGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAG CAGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACA ACCACTACACCCAGAAGAGCCTGAGCCTGTCACCCGGCAAG
SEQ ID NO167	DNA Heavy Chain	CAGGTGCAGCTGGTGCAGAGCGGAGCTGAGGTGAAGAAGCCAGGCGCCAGCGTCAA GGTGTCCTGCAAGGCCAGCGGCTACACCTTCACCAGCAGCTACATCAACTGGGTGCG CCAGGCTCCAGGGCAGGGACTGGAGTGGATGGGCCAGATCAACGCCGCCAGCGGC ATGACCAGATACGCCCAGAAGTTCCAGGGCAGAGTCACAATGACCAGGGACACCTCT ATCAGCACCGCCTACATGGAGCTGTCCAGGCTGAGAAGCGACGACACCGCCGTGTA CTACTGCGCCAGGGGCGGCTGGTTCGACTACTGGGGCCAGGGCACCCTGGTGACCG TGTCCTCAGCTAGCACCAAGGGCCCCAGCGTGTTCCCCCTGGCCCCCAGCAGCAAG AGCACCTCCGGCGGCACAGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGA GCCCGTGACCGTGTCCTGGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTTCC CCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGTCCAGCGTGGTGACAGTGCCC AGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAAC ACCAAGGTGGACAAGAGAGTGGAGCCCAAGAGCTGCGACAAGACCCACACCTGCCC CCCCTGCCCAGCCCCCGAAGCTGCAGGCGGCCCTTCCGTGTTCCTGTTCCCCCCCA AGCCCAAGGACACCCTGATGATCAGCAGGACCCCCGAGGTGACCTGCGTGGTGGTG GACGTGAGCCACGAGGACCCAGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGA GGTGCACAACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACAGGG

SEQ ID NO	Ab region	Sequence
		TGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGT GCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAAAAGACCATCAGCAAGGCCA AGGGCCAGCCACGGGAGCCCCAGGTGTACACCCTGCCCCCTTCTCGGGAGGAGATG ACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATC GCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCC AGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGAG CAGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACA ACCACTACACCCAGAAGAGCCTGAGCCTGTCACCCGGCAAG
SEQ ID NO168	DNA Heavy Chain	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCAATATTAATGCTGCTGCTGGTATTA CTCTTTATGCTCAGAAGTTTCAGGGTCGGGTCACCATGACCCGTGATACCAGCATTAG CACCGCGTATATGGAACTGAGCCGCCTGCGTAGCGATGATACGGCCGTGTATTATTG CGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCTC AGCCTCCACCAAGGGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTC TGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGA CGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTC CTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGC TTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTG GACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCA GCACCTGAAGCAGCGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGA CACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCC ACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG CCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTC CTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC AACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC CGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCA GGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACT CCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGC AGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGC AGAAGAGCCTCTCCCTGTCTCCGGGTAAA
SEQ ID NO169	DNA Heavy Chain	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCGGTATTAATCCTCCTGCTGGTACTA CTTCTTATGCTCAGAAGTTTCAGGGTCGGGTCACCATGACCCGTGATACCAGCATTAG CACCGCGTATATGGAACTGAGCCGCCTGCGTAGCGATGATACGGCCGTGTATTATTG CGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCTC AGCCTCCACCAAGGGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTC TGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGA CGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTC CTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGC TTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTG GACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCA GCACCTGAAGCAGCGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGA CACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCC ACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG CCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTC CTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC AACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC CGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCA

SEQ ID NO	Ab region	Sequence
		GGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACT CCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGC AGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGC AGAAGAGCCTCTCCCTGTCTCCGGGTAAA
SEQ ID NO170	DNA Heavy Chain	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCAATATTAATCCTGCTACTGGTCATG CTGATTATGCTCAGAAGTTTCAGGGTCGGGTGACCATGACCCGTGATACCAGCATTA GCACCGCGTATATGGAACTGAGCCGCCTGCGTAGCGATGATACGGCCGTGTATTATT GCGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCT CAGCCTCCACCAAGGGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCT CTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGT CCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAG CTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGT GGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCC AGCACCTGAAGCAGCGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGA CACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCC ACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG CCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTC CTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC AACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC CGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCA GGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACT CCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGC AGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGC AGAAGAGCCTCTCCCTGTCTCCGGGTAAA
SEQ ID NO171	DNA Light Chain	CAGAGCGCCCTGACCCAGCCCGCCAGCGTGTCCGGCAGCCCAGGCCAGTCTATCAC AATCAGCTGCACCGGCACCTCCAGCGACGTGGGCAGCTACAACTACGTGAACTGGTA TCAGCAGCACCCCGGCAAGGCCCCCAAGCTGATGATCTACGGCGTGAGCAAGAGGC CCAGCGGCGTGTCCAACAGGTTCAGCGGCAGCAAGAGCGGCAACACCGCCAGCCTG ACAATCAGTGGGCTGCAGGCTGAGGACGAGGCCGACTACTACTGCGGCACCTTTGC CGGCGGATCATACTACGGCGTGTTCGGCGGAGGGACCAAGCTGACCGTGCTGGGCC AGCCTAAGGCTGCCCCCAGCGTGACCCTGTTCCCCCCCAGCAGCGAGGAGCTGCAG GCCAACAAGGCCACCCTGGTGTGCCTGATCAGCGACTTCTACCCAGGCGCCGTGAC CGTGGCCTGGAAGGCCGACAGCAGCCCCGTGAAGGCCGGCGTGGAGACCACCACC CCCAGCAAGCAGAGCAACAACAAGTACGCCGCCAGCAGCTACCTGAGCCTGACCCC CGAGCAGTGGAAGAGCCACAGGTCCTACAGCTGCCAGGTGACCCACGAGGGCAGCA CCGTGGAAAAGACCGTGGCCCCAACCGAGTGCAGC
SEQ ID NO172	DNA Light Chain	CAGAGCGCCCTGACCCAGCCCGCCAGCGTGTCCGGCAGCCCAGGCCAGTCTATCAC AATCAGCTGCACCGGCACCTCCAGCGACGTGGGCAGCTACAACTACGTGAACTGGTA TCAGCAGCACCCCGGCAAGGCCCCCAAGCTGATGATCTACGGCGTGAGCAAGAGGC CCAGCGGCGTGTCCAACAGGTTCAGCGGCAGCAAGAGCGGCAACACCGCCAGCCTG ACAATCAGTGGGCTGCAGGCTGAGGACGAGGCCGACTACTACTGCGGCACCTTTGC CGGCGGATCATACTACGGCGTGTTCGGCGGAGGGACCAAGCTGACCGTGCTGGGCC AGCCTAAGGCTGCCCCCAGCGTGACCCTGTTCCCCCCCAGCAGCGAGGAGCTGCAG

SEQ ID NO	Ab region	Sequence
		GCCAACAAGGCCACCCTGGTGTGCCTGATCAGCGACTTCTACCCAGGCGCCGTGAC CGTGGCCTGGAAGGCCGACAGCAGCCCCGTGAAGGCCGGCGTGGAGACCACCACC CCCAGCAAGCAGAGCAACAACAAGTACGCCGCCAGCAGCTACCTGAGCCTGACCCC CGAGCAGTGGAAGAGCCACAGGTCCTACAGCTGCCAGGTGACCCACGAGGGCAGCA CCGTGGAAAAGACCGTGGCCCCAACCGAGTGCAGC
SEQ ID NO173	DNA Light Chain	CAGAGCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTAC CATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTAC CAGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCC TCAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGAC CATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGG TGGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCAGCC CAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAA CAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGC CTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTCCA AACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCCTGAGCAGT GGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAG AAGACAGTGGCCCCTACAGAATGTTCA
SEQ ID NO174	DNA Light Chain	CAGAGCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTAC CATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTAC CAGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCC TCAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGAC CATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGG TGGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCAGCC CAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAA CAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGC CTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTCCA AACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCCTGAGCAGT GGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAG AAGACAGTGGCCCCTACAGAATGTTCA
SEQ ID NO175	DNA Light Chain	CAGAGCGCACTGACCCAGCCAGCTTCAGTGAGCGGCTCACCAGGTCAGAGCATTAC CATCTCGTGTACGGGTACTAGCAGCGATGTTGGTTCTTATAATTATGTGAATTGGTAC CAGCAGCATCCCGGGAAGGCGCCGAAACTTATGATTTATGGTGTTTCTAAGCGTCCC TCAGGCGTGAGCAACCGTTTTAGCGGATCCAAAAGCGGCAACACCGCGAGCCTGAC CATTAGCGGCCTGCAAGCGGAAGACGAAGCGGATTATTATTGCGGTACTTTTGCTGG TGGTTCTTATTATGGTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTCAGCC CAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAA CAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGC CTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTCCA AACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCCTGAGCAGT GGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAG AAGACAGTGGCCCCTACAGAATGTTCA
SEQ ID NO176	DNA Heavy Chain	CAGGTGCAGCTGGTGCAGAGCGGAGCTGAGGTGAAGAAGCCAGGCGCCAGCGTCAA GGTGTCCTGCAAGGCCAGCGGCTACACCTTCACCAGCAGCTACATCAACTGGGTCCG CCAGGCTCCTGGGCAGGGACTGGAGTGGATGGGCACCATCAACCCCGTGTCCGGCA GCACCAGCTACGCCCAGAAGTTCCAGGGCAGAGTCACCATGACCAGGGACACCAGC ATCAGCACCGCCTACATGGAGCTGTCCAGGCTGAGAAGCGACGACACCGCCGTGTA

SEQ ID NO	Ab region	Sequence
		CTACTGCGCCAGGGGCGGCTGGTTCGACTACTGGGGCCAGGGCACCCTGGTGACCG TGTCCTCAGCTAGCACCAAGGGCCCCAGCGTGTTCCCCCTGGCCCCCTGCAGCAGA AGCACCAGCGAGAGCACAGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGA GCCAGTGACCGTGTCCTGGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTTCC CCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGTCCAGCGTGGTGACCGTGCCC AGCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTGGACCACAAGCCCAGCAAC ACCAAGGTGGACAAGACCGTGGAGAGGAAGTGCTGCGTGGAGTGCCCCCCCTGCCC AGCCCCCCCAGTGGCCGGACCCTCCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACA CCCTGATGATCAGCAGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCAC GAGGACCCAGAGGTGCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGC CAAGACCAAGCCCAGAGAGGAACAGTTTAACAGCACCTTCAGGGTGGTGTCCGTGCT GACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCA ACAAGGGCCTGCCAGCCCCCATCGAGAAAACCATCAGCAAGACCAAGGGCCAGCCA CGGGAGCCCCAGGTGTACACCCTGCCCCCCAGCCGGGAGGAAATGACCAAGAACCA GGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGT GGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCCCATGCTGGAC AGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGACAGTGGACAAGAGCAGGTGGCA GCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACA CCCAGAAGAGCCTGAGCCTGTCCCCCGGCAAG
SEQ ID NO177	DNA Heavy Chain	CAGGTGCAGCTGGTGCAGAGCGGAGCTGAGGTGAAGAAGCCAGGCGCCAGCGTCAA GGTGTCCTGCAAGGCCAGCGGCTACACCTTCACCAGCAGCTACATCAACTGGGTGCG CCAGGCTCCAGGGCAGGGACTGGAGTGGATGGGCCAGATCAACGCCGCCAGCGGC ATGACCAGATACGCCCAGAAGTTCCAGGGCAGAGTCACAATGACCAGGGACACCTCT ATCAGCACCGCCTACATGGAGCTGTCCAGGCTGAGAAGCGACGACACCGCCGTGTA CTACTGCGCCAGGGGCGGCTGGTTCGACTACTGGGGCCAGGGCACCCTGGTGACCG TGTCCTCAGCTAGCACCAAGGGCCCCAGCGTGTTCCCCCTGGCCCCCTGCAGCAGA AGCACCAGCGAGAGCACAGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGA GCCAGTGACCGTGTCCTGGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTTCC CCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGTCCAGCGTGGTGACCGTGCCC AGCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTGGACCACAAGCCCAGCAAC ACCAAGGTGGACAAGACCGTGGAGAGGAAGTGCTGCGTGGAGTGCCCCCCCTGCCC AGCCCCCCCAGTGGCCGGACCCTCCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACA CCCTGATGATCAGCAGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCAC
		GAGGACCCAGAGGTGCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGC CAAGACCAAGCCCAGAGAGGAACAGTTTAACAGCACCTTCAGGGTGGTGTCCGTGCT GACCGTGGTGCACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCA ACAAGGGCCTGCCAGCCCCCATCGAGAAAACCATCAGCAAGACCAAGGGCCAGCCA CGGGAGCCCCAGGTGTACACCCTGCCCCCCAGCCGGGAGGAAATGACCAAGAACCA GGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGT GGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCCCATGCTGGAC AGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGACAGTGGACAAGAGCAGGTGGCA GCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACA CCCAGAAGAGCCTGAGCCTGTCCCCCGGCAAG
SEQ ID NO178	DNA Heavy Chain	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCAATATTAATGCTGCTGCTGGTATTA CTCTTTATGCTCAGAAGTTTCAGGGTCGGGTCACCATGACCCGTGATACCAGCATTAG CACCGCGTATATGGAACTGAGCCGCCTGCGTAGCGATGATACGGCCGTGTATTATTG

SEQ ID NO	Ab region	Sequence
		CGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCTC AGCTTCCACCAAGGGCCCCAGCGTGTTCCCCCTGGCCCCCTGCAGCAGAAGCACCA GCGAGAGCACAGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCCGTG ACCGTGAGCTGGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTTCCCCGCCGT GCTGCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTGACCGTGCCCAGCAGCA ACTTCGGCACCCAGACCTACACCTGCAACGTGGACCACAAGCCCAGCAACACCAAGG TGGACAAGACCGTGGAGCGGAAGTGCTGCGTGGAGTGCCCCCCCTGCCCTGCCCCT CCTGTGGCCGGACCCTCCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATG ATCAGCCGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCC CGAGGTGCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCA AGCCCCGGGAGGAACAGTTCAACAGCACCTTCCGGGTGGTGTCCGTGCTGACCGTG GTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGG CCTGCCTGCCCCCATCGAGAAAACCATCAGCAAGACAAAGGGCCAGCCCAGGGAAC CCCAGGTGTACACCCTGCCCCCCAGCCGGGAGGAAATGACCAAGAACCAGGTGTCC CTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAG CAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCCCATGCTGGACAGCGACG GCAGCTTCTTCCTGTACAGCAAGCTGACAGTGGACAAGAGCCGGTGGCAGCAGGGC AACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAG AGCCTGAGCCTGTCCCCCGGCAAA
SEQ ID NO179	DNA Heavy Chain	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCGGTATTAATCCTCCTGCTGGTACTA CTTCTTATGCTCAGAAGTTTCAGGGTCGGGTCACCATGACCCGTGATACCAGCATTAG CACCGCGTATATGGAACTGAGCCGCCTGCGTAGCGATGATACGGCCGTGTATTATTG CGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCTC AGCTTCCACCAAGGGCCCCAGCGTGTTCCCCCTGGCCCCCTGCAGCAGAAGCACCA GCGAGAGCACAGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCCGTG ACCGTGAGCTGGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTTCCCCGCCGT GCTGCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTGACCGTGCCCAGCAGCA ACTTCGGCACCCAGACCTACACCTGCAACGTGGACCACAAGCCCAGCAACACCAAGG TGGACAAGACCGTGGAGCGGAAGTGCTGCGTGGAGTGCCCCCCCTGCCCTGCCCCT CCTGTGGCCGGACCCTCCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATG ATCAGCCGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCC CGAGGTGCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCA AGCCCCGGGAGGAACAGTTCAACAGCACCTTCCGGGTGGTGTCCGTGCTGACCGTG
		GTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGG CCTGCCTGCCCCCATCGAGAAAACCATCAGCAAGACAAAGGGCCAGCCCAGGGAAC CCCAGGTGTACACCCTGCCCCCCAGCCGGGAGGAAATGACCAAGAACCAGGTGTCC CTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAG CAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCCCATGCTGGACAGCGACG GCAGCTTCTTCCTGTACAGCAAGCTGACAGTGGACAAGAGCCGGTGGCAGCAGGGC AACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAG AGCCTGAGCCTGTCCCCCGGCAAA
SEQ ID NO180	DNA Heavy Chain	CAGGTGCAATTGGTTCAGAGCGGCGCGGAAGTGAAAAAACCGGGCGCGAGCGTGAA AGTGAGCTGCAAAGCCTCCGGATATACCTTTACTTCTTCTTATATTAATTGGGTCCGCC AAGCCCCTGGGCAGGGTCTCGAGTGGATGGGCAATATTAATCCTGCTACTGGTCATG CTGATTATGCTCAGAAGTTTCAGGGTCGGGTGACCATGACCCGTGATACCAGCATTA GCACCGCGTATATGGAACTGAGCCGCCTGCGTAGCGATGATACGGCCGTGTATTATT GCGCGCGTGGTGGTTGGTTTGATTATTGGGGCCAAGGCACCCTGGTGACGGTTAGCT

SEQ ID NO	Ab region	Sequence
		CAGCTTCCACCAAGGGCCCCAGCGTGTTCCCCCTGGCCCCCTGCAGCAGAAGCACC AGCGAGAGCACAGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCCGT GACCGTGAGCTGGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTTCCCCGCCG TGCTGCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTGACCGTGCCCAGCAGC AACTTCGGCACCCAGACCTACACCTGCAACGTGGACCACAAGCCCAGCAACACCAAG GTGGACAAGACCGTGGAGCGGAAGTGCTGCGTGGAGTGCCCCCCCTGCCCTGCCCC TCCTGTGGCCGGACCCTCCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGAT GATCAGCCGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGAC CCCGAGGTGCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGAC CAAGCCCCGGGAGGAACAGTTCAACAGCACCTTCCGGGTGGTGTCCGTGCTGACCG TGGTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGG GCCTGCCTGCCCCCATCGAGAAAACCATCAGCAAGACAAAGGGCCAGCCCAGGGAA CCCCAGGTGTACACCCTGCCCCCCAGCCGGGAGGAAATGACCAAGAACCAGGTGTC CCTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGA GCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCCCATGCTGGACAGCGAC GGCAGCTTCTTCCTGTACAGCAAGCTGACAGTGGACAAGAGCCGGTGGCAGCAGGG CAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAA GAGCCTGAGCCTGTCCCCCGGCAAA
SEQ ID NO181	ActRIIB	MTAPWVALALLWGSLCAGSGRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLH CYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHL PEAGGPEVTYEPPPTAPTLLTVLAYSLLPIGGLSLIVLLAFWMYRHRKPPYGHVDIHEDPG PPPPSPLVGLKPLQLLEIKARGRFGCVWKAQLMNDFVAVKIFPLQDKQSWQSEREIFSTP GMKHENLLQFIAAEKRGSNLEVELWLITAFHDKGSLTDYLKGNIITWNELCHVAETMSRGL SYLHEDVPWCRGEGHKPSIAHRDFKSKNVLLKSDLTAVLADFGLAVRFEPGKPPGDTHG QVGTRRYMAPEVLEGAINFQRDAFLRIDMYAMGLVLWELVSRCKAADGPVDEYMLPFEE EIGQHPSLEELQEVVVHKKMRPTIKDHWLKHPGLAQLCVTIEACWDHDAEARLSAGCVEE RVSLIRRSVNGTTSDCLVSLVTSVTNVDLPPKESSI
SEQ ID NO182	ActRIIB ligand binding domain (aa19-134)	SGRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGC WLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT
SEQ ID NO183	Antibody binding region	IELVKKGSWLDDFNS
SEQ ID NO184	Antibody binding region	VKKGSWLDDFNSYDR
SEQ ID NO185	Antibody binding region	GSWLDDFNSYDRQES
SEQ ID NO186	Antibody binding region	GCWLDDFNC
SEQ ID	Antibody binding	CEGEQDKRLHCYASW

SEQ ID NO	Ab region	Sequence
NO187	region	
SEQ ID NO188	Antibody binding region	WLDDFN
SEQ ID NO189	Antibody binding region	EQDKR
SEQ ID NO190	Antibody binding region	KGCWLDDFNCY
SEQ ID NO191	Antibody binding region	CIYYNANWELERT
SEQ ID NO192	Antibody binding region	YFCCCEGNFCN
SEQ ID NO193	Light - h/mIgG2 aLALA Chain	DIALTQPASVSGSPGQSITISCTGTSSDVGSYNYVNWYQQHPGKAPKLMIYGVSKRPSGV SNRFSGSKSGNTASLTISGLQAEDEADYYCGTFAGGSYYGVFGGGTKLTVLGQPKSTPTL TVFPPSSEELKENKATLVCLISNFSPSGVTVAWKANGTPITQGVDTSNPTKEGNKFMASS FLHLTSDQWRSHNSFTCQVTHEGDTVEKSLSPAECL
SEQ ID NO194	Heavy-h/mIgG2 aLALA chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFT SSYINWVRQAPGQGLEWM GTINPVSGSTSYAQKFQGRVTMTRDTSISTAYMELSSLRSEDTAVYYCARGGWFDYWGQ GTLVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHT FPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCP APNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQT HREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVL PPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKL RVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

[0200] The invention having been fully described is further illustrated by the following examples which are illustrative and are not meant to be further limiting.

EXAMPLES

General Methodology

[0201] ActRIIB antibodies, their characterisation and methods related thereto like (i) Functional

Assays, (ii) REPORTER GENE ASSAYs (RGA), (iii) Cultivation of HEK293T/17 Cell Lines, (iv) Myostatin-Induced Luciferase Reporter Gene Assays,(v) SPECIFICITY ELISAs, (vi) ActRIIB/Fc-Myostatin Binding Interaction ELISA, (vii) FACS titration on hActRIIB- and hActRIIA-Expressing Cells, (viii) Binding to primary human skeletal muscle cells, (ix) affinity Determination of Selected Anti-Human ActRIIB Fabs Using Surface Plasmon Resonance (Biacore), (x) CK ASSAY, (xi) Animal Models, (xii) TREATMENT PROTOCOLS, (xiii) Statistical Analysis, (xiiii) Pannings, (xv)antibody identification and characterization, (xvi) Optimization of antibodies derived from first affinity maturation, (xvii) IgG2 Conversion of Affinity Matured Fabs (1st Maturation), (xviiii) Second Affinity Maturation, (xx) IgG2 Conversion and Characterization of IgG2 (2nd Maturation), (xxi) Characterization of anti-ActRIIB antibodies in in vivo murine studies, (xxii) Confirmation of affinity by SET, (xxiii) Cross Blocking Studies and (xxiv) Epiotpe mapping details and technologies have been disclosed in the WO 2010/125003.

Working Examples: molecular biology

[0202] *Brown adipocyte differentiation.* Primary brown pre-adipocytes were isolated from interscapular brown adipose tissue of 5 week old male C57BL6/J mice using collagenase dissociation as described previously (Feige, et al 2008). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (Sigma), 3nM insulin (Sigma) and an antibiotic cocktail (Invitrogen). After reaching confluency, brown pre-adipocytes were differentiated in 12 well plates during 9 days using 20nM insulin and 1nM tri-iodo-thyronine (T3; Sigma) for the entire protocol and 0.5mM IBMX (Sigma), 0.5µM dexamethasone (Sigma), and 0.125mM indomethacin (Sigma), for the first two days. Medium and treatments were replaced every 2 days.

[0203] *Animal experiments.* Mouse experiments were performed in accordance with the Swiss ordinance on animal experimentation after approval by cantonal veterinarian authorities. Ten-week-old male C57Bl6/J male mice (Janvier laboratories, France) or CB17/ICR-*Prkdc*^{scid}/Crl female mice (Scid mice; Charles River, Germany) were maintained at 22°C in a 12 hour light-dark cycle with unrestricted access to regular diet and water. Animals were treated with the antibodies for four weeks by weekly sub-cutaneous injection at a volume of 5mL/kg and a dose of 20mg/kg, unless otherwise stated. Cold tolerance was evaluated by placing animals for 4 hours at 10°C in individual cages and measuring body temperature every hour with a rectal thermometer (Bioseb). All animals were sacrificed with CO_2 and the cold challenged animals were sacrificed right after the 24h cold exposure.

[0204] *Gene expression profiling.* RNA is extracted using Trizol reagent (Invitrogen). Reverse transcription was performed with random hexamers on 1µg of total RNA using a high capacity reverse transcription kit (Applied Biosystems) and the reaction was diluted 100 times for amplification. PCR reactions were performed in duplicate in 384-well plates on a CFX384 cycler (BioRad) using specific taqman probes (Applied Biosystems). Data were normalized to one or two house-keeping genes using the ΔΔCt method.

[0205] *Hematological profiling in non-human primates.* MOR08159 was administered once a week for 3 months to male and female cynomolgus monkeys by intravenous injection. 32 cynomolgus monkeys (16/sex) were assigned to one of four treatment groups (3 to 5 animals/sex/group) and were administered intravenous injections of either vehicle or MOR08159 at 10, 30, or 100 mg/kg once weekly for 13 weeks (total of 14 doses). Parameters evaluated included general clinical pathology (hematology, clinical chemistry, and coagulation). MOR08159 caused no significant hematological changes throughout the study in red blood cell, red blood cell distribution width, mean corpuscular hemoglobin concentration in male and female monkeys (see table 2 for male data).

[0206] *Hematological profiling in healthy volunteers.* MOR08159 was administered as a single intravenous infusion of 0 (placebo), 0.1, 0.3, 1, 3, 10, or 30 mg/kg to male and female healthy volunteers. Females were confirmed as either postmenaposal or surgically sterile prior to infusion. Hematological parameters including red blood cell counts were evaluated pre dosing as baseline and at 4 and 8 weeks post dosing. MOR08159 caused no significant changes in red blood cell parameters compared to placebo throughout the study period (see table 3).

Results:

Effect on brown adipose tissue and hematological parameters.

[0207] Inhibition of ActRIIB signaling pathway by an ActRIIB antibody (Ab) dramatically enhances the differentiation of primary brown adipocytes as reflected by an increase in thermogenic genes such as UCP-1. In contrast, myostatin, an ActRIIB ligand, is able to inhibit the differentiation of primary brown adipocytes, an effect which can be prevented by the administration of an ActRIIB pathway inhibitor such as ActRIIB Ab (figure 1).

[0208] Inhibition of ActRIIB signaling via administration of an ActRIIB Ab for 4 weeks to naive mice significantly increased not only skeletal muscle mass but also interscapular brown fat while no significantly changes in white fat were detected (figure 2).

[0209] The influence of ActRIIB inhibition on the functionality of brown adipose tissue was evaluated in vivo by challenging mice through cold exposure. Mice treated with the ActRIIB antibody for four weeks were significantly protected from hypothermia upon cold exposure when compared to vehicle treated mice (figure 3). This demonstrated that ActRIIB inhibition enhances adaptive thermogenesis.

[0210] This functional protection could, at least in part be accounted for by an increase in cellular respiration in primary brown adipocytes treated with an ActRIIBAb (figure 3. Importantly, the pharmacological inhibition of ActRIIB signaling using an ActRIIB antibody resulted in an increased amount of brown fat in adult animals, and translated into enhanced

energy expenditure and thermogenesis (figures 2 and 3).

[0211] ActRIIB inhibition brought about by weekly administration of an ActRIIB Antibody over 13 weeks (total 14 doeses) in cynomolgus monkeys does caused no significant hematological change throughout the study in red blood cell, red blood cell distribution width, and mean corpuscular hemoglobin concentration (see table 2). Similarly, single intravenous administration of ActRIIB Ab in human healthy volunteers up to 30 mg/kg did not induced any significant changes in red blood cells/hematological parameters up to 10 weeks post dosing (see table 3).

Table 2: Hematalogical parameters after 13 weeks of MOR08159 application in cynomolgus monkeys (E3= 10^3 and E6= 10^6)

		Dose (mg/kg) i.v.			
		0 (n=5)	10 (n=3)	30 (n=3)	100 (n=5)
RBC (E6/ul)	Pre-dosing	5.97±0.388	5.98±0.255	5.70±0.353	5.74±0.161
RBC (E6/ul)	Day 92	5.97±0.536	5.77±0.206	5.27±0.215	5.55±0.151
MCHC (g/dL)	Pre-dosing	30.2±1.65	30.1±0.55	29.9±1.46	31.4±1.34
MCHC (g/dL)	Day 92	28.8±1.59	30.1±0.40	29.4±0.40	30.4±1.07
RDW (%)	Pre-dosing	12.6±0.79	11.9±0.78	12.1±1.27	11.9±0.59
RDW (%)	Day 92	12.8±0.53	11.6±0.67	12.0±1.06	12.1±0.43

Table 3: Red blood cells counts after single injection of MOR08159 in human healthy volunteers (E6=10^6)

	RBC (E6/ul)		
	Pre-dosing (Bas)	Week 4 (d29)	Week 8 (d57)
Placebo (n=12)	4.87±0.45	4.85±0.51	4.81±0.35
1 mg/kg (n=6)	5.05±0.24	4.88±0.36	5.03±0.26
3 mg/kg (n=6)	5.22±0.41	5.12±0.22	5.40±0.24
10 mg/kg (n=6)	5.12±0.38	5.07±0.51	5.05±0.44
30 mg/kg (n=6)	5.12±0.29	5.12±0.27	5.17±0.31

Effect on metabolic parameters and brown adipocytes in mice fed a high-fat diet.

[0212] Effect of inhibition of ActRIIB signaling pathway by an ActRIIB antibody in mice fed a high-fat diet can be investigated in a therapeutic modality. Mice displaying some metabolic

dysfunction consuming a high-fat diet for several weeks are treated with an ActRIIB antibody or matched vehicle to examine the metabolic benefit on blood parameters, glucose and insulin levels, tissue distribution, thermogenic brown adipose tissue in particular, and tissue specific gene signature.

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PATENTKRAV

1. Sammensætning omfattende et antagonist-antistof, som binder til ActRIIB til anvendelse ved behandlingen af en metabolisk lidelse i et subjekt, hvor anti-ActRIIB-antistoffet forøger brunt adipost væv uden at forøge niveauet af røde blodceller i subjektet, og hvor anti-ActRIIB-antistoffet omfatter en tungkæde-variabel region CDR1 ifølge SEQ ID NO: 9; en tungkæde-variabel region CDR2 ifølge SEQ ID NO: 23; en tungkæde-variabel region CDR3 ifølge SEQ ID NO: 37; en letkæde-variabel region CDR1 ifølge SEQ ID NO: 51; en letkæde-variabel region CDR2 ifølge SEQ ID NO: 65; og en letkæde-variabel region CDR3 ifølge SEQ ID NO: 79, hvor den metaboliske lidelse er valgt fra gruppen bestående af obesitas, type 2 diabetes, metabolisk syndrom, lipodystrofi, forringet glucosetolerance, forhøjet plasmainsulinkoncentration, insulinresistens, dyslipidæmi, hyperglykæmi, hyperlipidæmi, hypertension, kardiovaskulær lidelse.

2. Sammensætning ifølge krav 1, hvor den metaboliske lidelse resulterer i eller er forårsaget af en forøget gennemsnitlig plasmaglucosekoncentration, unormal glucosehomeostase og/eller forhøjet plasmainsulinkoncentration.

3. Sammensætning ifølge krav 1 til 2, hvor subjektet lider af en metabolisk lidelse og en muskellidelse.

4. Sammensætning ifølge krav 3, hvor muskellidelsen er en muskelatrofi valgt fra gruppen bestående af obesitas-associteret sarcopeni, sarcopeni og diabetesassocieret muskelatrofi.

5. Sammensætning ifølge ethvert af de foregående krav, hvor antistoffet binder til ActRIIB med en 10-gange større affinitet end det binder til ActRIIA.

6. Sammensætning ifølge ethvert af de foregående krav, hvor anti-ActRIIB-antistoffet omfatter tungkædesekvensen ifølge SEQ ID NO: 146 og letkædesekvensen ifølge SEQ ID NO: 141;

7. Sammensætning ifølge ethvert af de foregående krav, hvor anti-ActRIIB-antistoffet indeholdt i sammensætningen har ændret effektorfunktion via mutation af Fc-regionen.

8. Sammensætning ifølge ethvert af de foregående krav, hvor anti-ActRIIB-antistoffet indeholdt i sammensætningen er kodet for af pBW522 som DSM22873 eller pBW524 som DSM22874, deponeret ved DSMZ den 18.08.2009.